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# CLINICAL AND EXPERIMENTAL

## ON THE PROCESS OF LYMPH FORMATION*

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WE HAVE met no satisfactory theory on the process of lymph formation, though some theories have been published, such as the filtration theory of Ludwig,[1] the secretion theory of Heidenhain,[2] and the cellular physiologic theory of Asher.[3]

Lymphagogues as defined by Heidenhain[2] are substances which, injected into the blood stream, cause an increased flow of lymph from the thoracic duct. He divided them into two classes. The first class includes such substances as peptone, leech extract, crayfish extract, etc. These bodies cause an increased flow of more concentrated lymph. The second class includes such substances as sugar, salt, urea, etc. These increase the lymph flow, the lymph becoming more watery than before.

It will be seen that there is an obvious difference between the lymph produced by either class of lymphagogues; therefore, it is better to study their actions on the basis of the two classifications.

### THE SECOND CLASS OF LYMPHAGOGUES

Heidenhain[2] found that solutions of sodium chloride, sodium nitrate, sodium sulphate, and sodium iodide of the same percentage are not equally effective in increasing lymph flow; sodium chloride with the smallest molecular weight is the most effective, sodium nitrate less so, sodium sulphate still less, and sodium iodide with the largest molecular weight the least effective. He concluded, therefore, that the effects on increasing lymph flow are parallel to the water-attracting power of the solutions.

Using equimolecular solutions, instead of those of the same percentage, Heinz[4] found that sodium chloride and sodium iodide are equally effective.

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I have studied solutions of different members of the second class to determine whether they are equally effective in increasing lymph flow, whether their water-attracting power was the same.

In aqueous solution, sodium chloride and sodium iodide dissociate into two ions, sodium sulphate into three ions, and sodium phosphate into four ions, while glucose does not dissociate at all. Therefore the solutions of these substances will be nearly of the same osmotic pressure if their concentration is as follows.

| | | |
|---|---|---|
| Sodium chloride | ½ mol | 2.9 per cent |
| Sodium iodide | ½ mol | 7.4 per cent |
| Sodium sulphate (crystal) | ⅓ mol | 10.7 per cent |
| Sodium phosphate (crystal) | ¼ mol | 8.9 per cent |
| Glucose (anhydrous) | 0.92 mol | 16.6 per cent |

Injecting each of the above solutions, 5 c.c. per kg. of body weight, into the vein of dog, I observed the influence on the lymph flow and the urine output.

The lymph flowing out of the thoracic duct was put into graduated cylinders and measured every ten minutes. At the same time the urine was obtained by a catheter retained in the bladder.

To compare the effects of each solution, I have used the following proportion showing it by L.

$$L = \frac{\text{Lymph quantity in 30 minutes after the injection}}{\text{Lymph quantity in 30 minutes before the injection}}$$

TABLE I

Gray bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (6.6 c.c. in 55 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 1.30′–40′ | 2.3 | 0.8 | |
| 40′–50′ | 2.1 | 0.8 | |
| 50′–60′ | 2.0 | 0.8 | |
| 2. 0′–10′ | 2.0 | 0.8 | |
| 10′–20′ | 2.4 | 0.8 | |
| 20′–30′ | 1.7 | 0.8 | |
| 30′–40′ | 4.6 | 3.4 | 30′–31′15″ |
| 40′–50′ | 4.8 | 2.4 | Sodium chloride (2.9 per cent) |
| 50′–60′ | 4.6 | 0.8 | 27.5 c.c. intravenously |
| 3. 0′–10′ | 4.3 | 0.8 | |
| 10′–20′ | 4.1 | 1.0 | |
| 20′–30′ | 2.8 | 1.0 | |
| 30′–40′ | 3.3 | 1.0 | |
| 40′–50′ | 2.6 | 1.0 | |
| 50′–60′ | 2.7 | 1.2 | |
| 4. 0′–10′ | 2.2 | 1.0 | |
| 10′–20′ | 1.7 | 0.8 | |
| 20′–30′ | 1.6 | 0.8 | |

$$L = \frac{4.6 + 4.8 + 4.6}{2.0 + 2.4 + 1.7} = 2.29$$

In Tables I and II we see that ½ mol solution of sodium chloride and ⅓ mol solution of sodium sulphate are nearly equally effective in increasing lymph flow but that sodium sulphate is much more effective than sodium chloride in increasing urine output.

TABLE II

Piebald bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (6.6 c.c. in 55 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 11.25′–35′ | 2.2 | 0.8 | |
| 35′–45′ | 2.3 | 0.8 | |
| 45′–55′ | 2.2 | 0.8 | |
| 55′–65′ | 2.2 | 1.0 | |
| 12. 5′–15′ | 2.3 | 1.0 | |
| 15′–25′ | 2.1 | 1.0 | |
| 25′–35′ | 4.9 | 22.4 | 25′0″–26′15″ |
| 35′–45′ | 5.8 | 10.6 | Sodium sulphate (10.7 per cent) 27.5 c.c. |
| 45′–55′ | 4.3 | 6.4 | intravenously |
| 55′–65′ | 3.1 | 4.6 | |
| 1. 5′–15′ | 3.0 | 4.4 | |
| 15′–25′ | 2.1 | 2.8 | |
| 25′–35′ | 2.1 | 2.6 | |

$$L = \frac{4.9 + 5.8 + 4.3}{2.2 + 2.3 + 2.1} = 2.27$$

TABLE III

Yellow bitch, 6.8 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.2 c.c. in 68 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 12.10′–20′ | 2.8 | 2.4 | |
| 20′–30′ | 2.8 | 2.0 | |
| 30′–40′ | 2.4 | 2.2 | |
| 40′–50′ | 2.2 | 2.6 | |
| 50′–60′ | 2.1 | 2.4 | |
| 1. 0′–10′ | 2.2 | 2.2 | |
| 10′–20′ | 4.7 | 14.6 | 10′0″–11′15″ |
| 20′–30′ | 3.7 | 6.8 | Sodium phosphate (8.9 per cent) |
| 30′–40′ | 3.5 | 6.0 | 34 c.c. intravenously |
| 40′–50′ | 2.6 | 3.4 | |
| 50′–60′ | 2.2 | 2.2 | |
| 2. 0′–10′ | 1.9 | 2.4 | |
| 10′–20′ | 2.0 | 2.8 | |
| 20′–30′ | 1.9 | 2.2 | |

$$L = \frac{4.7 + 3.7 + 3.5}{2.2 + 2.1 + 2.2} = 1.83$$

TABLE IV

Black bitch, 7.8 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.4 c.c. in 78 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 12.10′–20′ | 3.2 | 0.8 | |
| 20′–30′ | 3.1 | 1.0 | |
| 30′–40′ | 3.2 | 0.8 | |
| 40′–50′ | 3.1 | 0.8 | |
| 50′–60′ | 3.2 | 1.0 | |
| 1. 0′–10′ | 3.1 | 0.9 | |
| 10′–20′ | 7.6 | 10.8 | 10′0″–11′30″ |
| 20′–30′ | 7.9 | 8.0 | Glucose (16.6 per cent) 39 c.c. |
| 30′–40′ | 4.8 | 3.2 | intravenously |
| 40′–50′ | 4.4 | 1.6 | |
| 50′–60′ | 3.3 | 1.2 | |
| 2. 0′–10′ | 2.9 | 1.2 | |
| 10′–20′ | 1.9 | 0.8 | |

$$L = \frac{7.6 + 7.9 + 4.8}{3.1 + 3.2 + 3.1} = 2.15$$

TABLE V

Yellow bitch, 8.6 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (10.3 c.c. in 86 c.c. of water).

| TIME | LYMPH C.C. | URINE C.C. | NOTICE |
|---|---|---|---|
| 2.45′–55′ | 3.5 | 3.2 | |
| 55′–65′ | 3.5 | 3.2 | |
| 3. 5′–15′ | 3.5 | 3.2 | |
| 15′–25′ | 3.5 | 3.2 | |
| 25′–35′ | 3.2 | 3.2 | |
| 35′–45′ | 3.0 | 3.2 | |
| 45′–55′ | 2.9 | 3.0 | |
| 55′–65′ | 6.2 | 10.0 | 55′0″–56′30″ |
| 4. 5′–15′ | 6.7 | 7.0 | Sodium iodide (7.4 per cent) 43 c.c. |
| 15′–25′ | 6.1 | 8.4 | intravenously |
| 25′–35′ | 4.4 | 7.4 | |
| 35′–45′ | 4.0 | 7.2 | |
| 45′–55′ | 4.0 | 7.2 | |
| 55′–65′ | 3.7 | 7.3 | |
| 5. 5′–15′ | 3.6 | 7.2 | |
| 15′–25′ | 3.9 | 6.5 | |
| 25′–35′ | 4.0 | 6.5 | |
| 35′–45′ | 3.4 | 6.3 | |
| 45′–55′ | 3.6 | 5.4 | |
| 55′–65′ | 3.3 | 4.9 | |

$$L = \frac{6.2 + 6.7 + 6.1}{3.2 + 3.0 + 2.9} = 2.08$$

The effects of glucose and sodium iodide are nearly the same as those of sodium chloride and sodium sulphate, but sodium phosphate is a little less effective than the others. This is probably due to the fact that sodium phosphate does not dissociate completely into 4 ions but rather partly into three, i.e., two Na′ and one $HPo_4''$. Therefore the ¼ mol solution of sodium phosphate is of less osmotic pressure than the other solutions.

In 11 cases the results were nearly the same, as we see in Table VI.

TABLE VI

| SOLUTION | SODIUM CHLORIDE | SODIUM SULPHATE | SODIUM PHOSPHATE | GLUCOSE | SODIUM IODIDE |
|---|---|---|---|---|---|
| L | 2.29 | | | | |
| | | 2.27 | | | |
| | 1.65 | 1.75 | | | |
| | | 2.15 | | | |
| | | | 1.83 | | |
| | | | 1.77 | | |
| | 2.06 | | 1.87 | | |
| | 2.01 | | | 1.89 | |
| | 1.97 | | | 2.15 | |
| | | | | 1.97 | 2.18 |
| | | | | | 2.08 |
| Average | 1.99 | 2.06 | 1.82 | 2.00 | 2.13 |

(------) shows that two kinds of the solutions were injected one after the other in the same dog.

The effects on increasing lymph flow are nearly the same in all these cases. The increase of urine output is, however, not the same in all cases. Sodium sulphate causes the greatest increase, glucose and sodium phosphate next, and sodium iodide and sodium chloride least.

I examined further with more concentrated solutions.

TABLE VII

Black bitch, 12.7 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (15.2 c.c. in 127 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 5.20′–30′ | 4.9 | 3.2 | |
| 30′–40′ | 4.9 | 3.2 | |
| 40′–50′ | 4.8 | 2.8 | |
| 50′–60′ | 4.6 | 3.2 | |
| 6. 0′–10′ | 4.0 | 3.0 | |
| 10′–20′ | 4.0 | 3.0 | |
| 20′–30′ | 10.1 | 14.0 | 20′0″–21′45″ |
| 30′–40′ | 12.3 | 4.6 | Sodium chloride (5.8 per cent) 63.5 c.c. |
| 40′–50′ | 13.4 | 3.4 | intravenously |
| 50′–60′ | 12.1 | 4.0 | |
| 7. 0′–10′ | 11.2 | 4.0 | |
| 10′–20′ | 6.6 | 4.0 | |
| 20′–30′ | 5.5 | 4.0 | |
| 30′–40′ | 5.0 | 5.0 | |
| 40′–50′ | 6.8 | 4.2 | |
| 50′–60′ | 5.7 | 2.6 | |
| 8. 0′–10′ | 5.3 | 3.0 | |
| 10′–20′ | 4.8 | 3.0 | |
| 20′–30′ | 5.0 | 2.8 | |
| 30′–40′ | 4.3 | 3.6 | |
| 40′–50′ | 4.1 | 3.4 | |
| 50′–60′ | 3.9 | 3.2 | |
| 9. 0′–10′ | 11.9 | 54.2 | 0′0″–1′45″ |
| 10′–20′ | 12.9 | 28.0 | Sodium sulphate (21.4 per cent) 63.5 c.c. |
| 20′–30′ | 11.7 | 19.2 | intravenously |
| 30′–40′ | 7.0 | 14.0 | |
| 40′–50′ | 5.2 | 10.4 | |
| 50′–60′ | 4.7 | 10.0 | |

$$L = \frac{10.1 + 12.3 + 13.4}{4.6 + 4.0 + 4.0} = 2.84 \text{ (sodium chloride)}$$

$$L = \frac{11.9 + 12.9 + 11.7}{4.3 + 4.1 + 3.9} = 2.97 \text{ (sodium sulphate)}$$

TABLE VIII

Piebald bitch, 6.8 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.1 c.c. in 68 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 12. 0′–10′ | 2.8 | 1.2 | |
| 10′–20′ | 2.6 | 1.4 | |
| 20′–30′ | 2.6 | 1.4 | |
| 30′–40′ | 3.0 | 1.4 | |
| 40′–50′ | 2.9 | 1.6 | |
| 50′–60′ | 3.1 | 1.4 | |
| 1. 0′–10′ | 10.0 | 8.8 | 0′0″–1′30″ |
| 10′–20′ | 13.6 | 5.0 | Sodium chloride (11.6 per cent) 34 c.c. |
| 20′–30′ | 10.4 | 4.8 | intravenously |
| 30′–40′ | 9.9 | 3.8 | |
| 40′–50′ | 7.9 | 3.2 | |
| 50′–60′ | 5.1 | 2.2 | |
| 2. 0′–10′ | 5.5 | 2.0 | |
| 10′–20′ | 6.3 | 2.0 | |
| 20′–30′ | 5.8 | 1.6 | |
| 30′–40′ | 5.0 | 2.4 | |
| 40′–50′ | 4.0 | 2.2 | |
| 50′–60′ | 3.8 | 1.4 | |

$$L = \frac{10.0 + 13.6 + 10.4}{3.0 + 2.9 + 3.1} = 3.77$$

We see that the effect of the second class of lymphagogues is greater in proportion to the osmotic pressure of the solution used. Therefore their action may be said to be a salt action, as Heinz[4] has stated.

Starling[5] and Cohnstein[6] explain the action of the second class of lymphagogues as follows: On the injection of the lymphagogues into the blood, the osmotic pressure of the circulating fluid is largely increased. As a result water is attracted from lymph and tissues into the blood by a process of osmosis until the osmotic pressure of the circulating blood is restored to normal. A condition of hydremic plethora is thereby produced, generally attended by a rise of pressure in the capillaries. This rise of pressure is proportional to the increase of the volume of the blood and, therefore, to the osmotic pressure of the solution injected. This rise of capillary pressure causes a great increase in the transudation of fluid from the capillaries, and consequently an increase in the lymph flow.

To fulfill this condition the vessel wall must be completely semipermeable and the substance injected not allowed to come out of the vessel into the lymph spaces. But as stated by Heidenhain and Cohnstein, the injected substance is found in the lymph in nearly the same amount as in the blood. Moreover, as Starling points out, it is not easy to explain the movement of the water in two opposite directions at the same time, i.e., its coming from the lymph spaces into the vessel to dilute the blood and its passing from the vessel into the lymph spaces by filtration.

I would therefore explain the action of the second class of lymphagogues as follows: A hypertonic solution injected into the blood will be diluted with water attracted into the vessels from the lymph spaces. At the same time the injected substance will be excreted in part by the kidneys and will in part pass into the lymph spaces. Arrived here in a concentrated state, it robs the tissue cells of water by osmosis so that the volume of the interstitial fluid is largely increased and the excess flows away into the lymphatics, thus giving rise to the largely increased lymph flow. Of course the substance in the lymph spaces will also be partly fixed by the tissue cells. Thus we see that the glomeruli and the blood capillaries act for the same purpose of regulating the constitution of the blood. The emigration of the injected substance into the lymph spaces from the blood vessels is not explained by such physical processes as filtration or osmosis. It may be due to the secretory action of the endothelial cells. Accordingly the second class of lymphagogues excites the secretory action of the endothelial cells of both glomeruli and blood capillaries.

I have pointed out that if the osmotic pressures of all the above solutions are equal, the effects upon the increasing lymph flow are the same, but those upon increasing urine output are different. The effect will be the most with sodium sulphate and least with sodium chloride. It is easy to see that sodium chloride is more fixed than sodium sulphate by tissue cells since the former gets more easily into the tissue cells because it is the more diffusible. The less diffusible substance must be more quickly excreted by the kidneys and in greater amount, while the more diffusible substance does not need to be excreted so rapidly, being fixed by tissue cells in greater quantity.

### THE FIRST CLASS OF LYMPHAGOGUES

Heidenhain[2] stated that substances of this class exert a specific influence on the endothelial cells, causing them to secrete an increased amount of lymph. Starling[5] explained their action by increased permeability of the capillaries. Asher[3] stated that the first class increases liver function and that increased lymph flow is caused by increased physiologic function of some organ.

No one has tested the action of this class on fluids placed in the body outside the blood vessels. I have observed the influence of peptone on Ringer's solution previously infused into the abdominal cavity, comparing the increase of lymph flow with that of cases in which peptone alone was injected without preliminary infusion of Ringer's solution.

The dose of Ringer's solution was 50 c.c. per kg. of body weight; its temperature was 38° C.

At first the effect of Ringer's solution alone on lymph flow and urine output was observed.

TABLE IX

Yellow bitch, 6.7 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (8.0 c.c. in 67 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 1.20′–30′ | 1.6 | 0.7 | |
| 30′–40′ | 1.7 | 0.7 | |
| 40′–50′ | 1.7 | 0.7 | |
| 50′–60′ | 1.5 | 0.7 | |
| 2. 0′–10′ | 1.7 | 0.7 | |
| 10′–20′ | 1.5 | 0.8 | |
| 20′–30′ | 1.5 | 0.6 | |
| 30′–40′ | 1.7 | 0.6 | 30′–34′ Ringer's solution |
| 40′–50′ | 2.1 | 1.0 | 340 c.c. intraperitoneally |
| 50′–60′ | 1.9 | 1.6 | |
| 3. 0′–10′ | 2.2 | 1.4 | |
| 10′–20′ | 2.4 | 0.8 | |
| 20′–30′ | 2.4 | 0.8 | |
| 30′–40′ | 2.2 | 0.8 | |
| 40′–50′ | 2.3 | 0.8 | |
| 50′–60′ | 2.3 | 1.4 | |
| 4. 0′–10′ | 2.3 | 1.4 | |
| 10′–20′ | 2.5 | 1.0 | |
| 20′–30′ | 2.6 | 1.2 | |
| 30′–40′ | 2.5 | 1.2 | |
| 40′–50′ | 2.9 | 1.2 | |
| 50′–60′ | 2.9 | 1.6 | |
| 5. 0′–10′ | 2.7 | 2.4 | |
| 10′–20′ | 3.0 | 2.2 | |
| 20′–30′ | 2.5 | 1.2 | |
| 30′–40′ | 2.5 | 1.6 | |
| 40′–50′ | 2.5 | 1.8 | |
| 50′–60′ | 2.5 | 1.6 | |

After infusion with Ringer's solution both the lymph flow and the urine output increased slightly and slowly. In 3 other cases the results were the same. On injecting peptone into the blood vessel, the lymph flow increases and the urine output in most cases first decreases then increases again, as shown in Table X.

Tables XI and XII represent 12 experiments; in 7 of which the increase of lymph flow was much more than the controls (Table X and 9 other experiments). In 4 others of the 12, the increase of lymph flow was nearly the same or only a little more than the controls; but the increase in urine output was much more than that in the controls. In only 1 case was neither the lymph flow nor the urine output more than in the controls.

TABLE X

Black bitch, 7.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (9.0 c.c. in 75 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 11.25′–35′ | 2.4 | 1.0 | |
| 35′–45′ | 2.6 | 1.2 | |
| 45′–55′ | 2.2 | 1.0 | |
| 55′–65′ | 2.3 | 1.0 | |
| 12. 5′–15′ | 2.3 | 1.0 | |
| 15′–25′ | 2.2 | 1.0 | 25′–27′ peptone (2 per cent) 37.5 c.c. |
| 25′–35′ | 8.3 | 0.2 | intravenously |
| 35′–45′ | 8.7 | 0.2 | |
| 45′–55′ | 5.7 | 1.4 | |
| 55′–65′ | 5.8 | 3.2 | |
| 1. 5′–15′ | 6.1 | 4.3 | |
| 15′–25′ | 4.3 | 1.6 | |
| 25′–35′ | 3.1 | 2.2 | |
| 35′–45′ | 3.2 | 2.2 | |
| 45′–55′ | 2.5 | 2.4 | |

$$L = \frac{8.3 + 8.7 + 5.7}{2.3 + 2.3 + 2.2} = 3.33$$

In the majority of 9 experiments the proportion L was between 2 and 3, the highest being 3.3 and the lowest 1.87.

TABLE XI

Piebald bitch, 6.0 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (7.2 c.c. in 60 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 11.45′–55′ | 1.2 | 0.6 | |
| 55′–65′ | 1.8 | 0.6 | |
| 12. 5′–15′ | 1.5 | 0.4 | |
| 15′–25′ | 1.5 | 0.4 | |
| 25′–35′ | 1.8 | 0.4 | |
| 35′–45′ | 1.9 | 0.4 | |
| 45′–55′ | 1.6 | 0.4 | |
| 55′–65′ | 1.5 | 0.4 | |
| 1. 5′–15′ | 1.0 | 0.4 | 5′–10′ Ringer's solution 300 c.c. |
| 15′–25′ | 1.1 | 0.4 | intraperitoneally |
| 25′–35′ | 1.0 | 0.4 | |
| 1.35′–45′ | 8.7 | 0.2 | 35′–37′ peptone (2 per cent) 30 c.c. |
| 45′–55′ | 10.0 | 0.2 | intravenously |
| 55′–65′ | 6.4 | 1.4 | |
| 2. 5′–15′ | 3.4 | 1.0 | |
| 15′–25′ | 3.5 | 1.0 | |
| 25′–35′ | 5.2 | 0.6 | |
| 35′–45′ | 5.2 | 0.4 | |

$$L = \frac{8.7 + 10.0 + 6.4}{1.0 + 1.1 + 1.0} = 8.09$$

Thus we see that peptone intravenously promotes the emigration of Ringer's solution from the abdominal cavity into the lymphatics.

For explanation we may assume the following as the action of peptone: (1) Peptone may act on the fluid in the abdominal cavity so that it can emigrate more easily into the lymphatics. (2) Peptone may act either on the wall of the lymphatics or on that of the blood vessels making it easier for the fluid to migrate into the lymphatic system.

TABLE XII

Yellow bitch, 8.7 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (10.4 c.c. in 87 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 1.50′–60′ | 3.2 | 0.8 | |
| 2. 0′–10′ | 3.1 | 0.8 | |
| 10′–20′ | 3.6 | 0.8 | |
| 20′–30′ | 3.1 | 1.0 | |
| 30′–40′ | 3.6 | 1.0 | 40′–48′ Ringer's solution |
| 40′–50′ | 2.8 | 1.2 | 440 c.c. intraperitoneally |
| 50′–60′ | 2.7 | 1.0 | |
| 3. 0′–10′ | 8.0 | 0.2 | 0′–2′ peptone (2 per cent) 43 c.c. |
| 10′–20′ | 5.9 | 0.5 | intravenously |
| 20′–30′ | 4.5 | 2.3 | |
| 30′–40′ | 4.4 | 4.6 | |
| 40′–50′ | 2.8 | 3.2 | |
| 50′–60′ | 2.5 | 2.2 | |
| 4. 0′–10′ | 2.3 | 5.0 | |
| 10′–20′ | 2.5 | 11.0 | |
| 20′–30′ | 2.4 | 20.0 | |
| 30′–40′ | 2.3 | 21.0 | |
| 40′–50′ | 2.2 | 11.0 | |
| 50′–60′ | 2.5 | 9.6 | |
| 5. 0′–10′ | 3.0 | 7.2 | |
| 10′–20′ | 2.8 | 9.2 | |

$$L = \frac{8.0 + 5.9 + 4.5}{3.6 + 2.8 + 2.7} = 2.02$$

TABLE XIII

Piebald bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (6.6 c.c. in 55 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | NOTICE |
|---|---|---|---|
| 11.55′–65′ | 3.0 | 0.6 | |
| 12. 5′–15′ | 3.0 | 0.6 | |
| 15′–25′ | 2.8 | 0.6 | |
| 25′–35′ | 2.9 | 0.6 | |
| 35′–45′ | 3.0 | 0.6 | |
| 45′–55′ | 3.3 | 0.6 | |
| 55′–65′ | 2.7 | 0.6 | |
| 1. 5′–15′ | 2.6 | 0.6 | 7′–11′ Ringer's solution 275 c.c. |
| 15′–25′ | 2.3 | 0.6 | and peptone (2 per cent) 55 c.c. |
| 25′–35′ | 2.1 | 0.6 | intraperitoneally |
| 35′–45′ | 2.1 | 0.6 | |
| 45′–55′ | 2.3 | 0.6 | |
| 55′–65′ | 2.0 | 0.6 | |
| 2. 5′–15′ | 2.6 | 0.6 | |
| 15′–25′ | 1.9 | 0.6 | |
| 25′–35′ | 2.4 | 0.6 | |

Taking these factors into consideration, I investigated whether or not peptone increases the lymph flow when infused into the abdominal cavity, mixed with the Ringer's solution. (See Table XIII.)

Neither the lymph flow nor the urine output increases, in comparison with cases in which Ringer's solution alone is infused into the abdominal cavity. Although peptone may indirectly facilitate passage of fluid from the abdominal cavity into the lymphatic system, it does not do so by acting directly on this fluid.

Then we must decide whether peptone acts on the wall of the lymph vessel or on that of the blood vessel. If the isotonic fluid injected into the abdominal cavity is absorbed entirely through the blood vessels, as Orlow[7] and Hamburger[8] have stated, peptone must act on the blood vessel so that the fluid once absorbed into the blood vessel passes again into the lymph spaces in greater quantity than before. If the resorption from the abdominal cavity is entirely due to the lymphatic system, as Cohnstein[9] postulates, peptone must act on the lymphatic vessel to promote the passage of the fluid directly into the lymphatic system from the abdominal cavity.

But it is not settled whether resorption from the abdominal cavity is entirely through the lymphatics or entirely through the blood vessels. It may occur rather through both. We have no experimental method for determining the action on the lymphatic system. But if peptone really acts on the blood vessels, an isotonic fluid which has been slowly infused directly into the blood vessels must also pass into the lymph spaces after the injection of peptone. Therefore I injected peptone into the right femoral vein, while at the same time the Ringer's solution was being infused very slowly into the left femoral vein (5 c.c. per kg. of body weight took twenty-five minutes). According to Orlow,[7] the resorption of an isotonic fluid from the abdominal cavity is not rapid, 1.7-2.0 c.c. being absorbed in an hour when 25-33 c.c. per kg. has been

TABLE XIV

Black bitch, 9.4 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (11.3 c.c. in 94 c.c. of water).

| TIME | LYMPH C.C. | URINE C.C. | NOTICE |
|---|---|---|---|
| 12.25′–35′ | 2.8 | 1.2 | |
| 35′–45′ | 2.9 | 1.2 | |
| 45′–55′ | 3.2 | 1.4 | |
| 55′–65′ | 3.0 | 1.2 | 0′–25′ Ringer's solution 47 c.c. |
| 1. 5′–15′ | 4.5 | 1.2 | in the left femoral vein. |
| 15′–25′ | 8.1 | 1.0 | |
| 25′–35′ | 6.8 | 2.6 | 5′–7′ peptone (2 per cent) 47 c.c. |
| 35′–45′ | 5.0 | 2.0 | in the right femoral vein |
| 45′–55′ | 3.9 | 1.4 | |
| 55′–65′ | 3.2 | 1.0 | |
| 2. 5′–15′ | 2.6 | 1.2 | |
| 15′–25′ | 2.6 | 1.2 | |
| 25′–35′ | 2.4 | 1.2 | |
| 35′–45′ | 2.3 | 1.0 | |
| 45′–55′ | 2.7 | 1.0 | |

$$L = \frac{4.5 + 8.1 + 6.8}{2.9 + 3.2 + 3.0} = 2.13$$

injected. Therefore not so much Ringer's solution was infused into the left femoral vein.

In this protocol (Table XIV) we see that the increase of lymph flow is not more than when peptone alone is injected into the vein. In 4 other experiments the results were nearly the same. Accordingly the action of peptone is not on the blood vessels. Thus we may conclude that peptone promotes the migration of the fluid from the abdominal cavity into the lymphatics, acting on their walls, unless it acts on the fluid after it has itself been altered in the body. We cannot decide here in which way peptone acts. But as peptone promotes the passage of fluid from the abdominal cavity it is easy to see that the migration of tissue fluid will also be promoted by peptone. This we assume is the cause of the increase of lymph flow by peptone injection.

I have mentioned before that in 4 out of 12 cases in which Ringer's solution had been previously injected into the abdominal cavity and then peptone injected, the lymph flow was not more, but that the urine output was much more, than in the controls. The great increasing of urine output in these 4 cases shows that the infused fluid went for the most part into the blood circulation and was excreted by the kidney. It seems that in these 4 animals resorption from the abdominal cavity was mainly through the blood vessels, with the result that lymph flow was not greater than in the controls.

It is well known that peptone injection increases the concentration of the blood. Underhill and Ringer[10] explained this phenomenon as follows: On injection of peptone, the permeability of blood capillaries increases, as Starling stated; and consequently, because of this increased permeability, more plasma goes into the lymph spaces, and the blood is more concentrated. But this might be a secondary phenomenon to the primary passage of tissue fluids into the lymphatic system.

### PITUITRIN

Numerous experiments on the influence of hypophyseal extract on the urine output have been carried out. The current opinion accepts its being first antidiuretic and then diuretic; yet divergences exist as to the cause of the diuresis.

Hoskins and Means[11] state that pituitrin excites secretion of the renal cells. King and Stoland[12] insist on dilatation of the kidney vessels. Pentimali and Querica[13] explain the diuresis from the point of blood pressure overcoming the constriction of kidney vessels and explain antidiuresis by the preliminary predominance of the latter. Veil,[14] Meyer and Meyer Bisch[15] claim that pituitrin concentrates the tissue fluids. The water liberated by the concentration passes into the blood vessels and is excreted through the kidneys. Especially Meyer and Meyer Bisch[15] insist on the concentration of the tissues, since they have demonstrated that on injection of pituitrin, a diminution of lymph flow and the presence of a more concentrated lymph result. Oehme[16] claims that concentration of the tissues cannot be proved, and according to his experiments pituitrin diuresis must be due to stimulation of the renal cells. Bauer and Aschner[17] also deny the primary action of pituitrin on the tissues. Using the blood concentration as a standard for comparison, Underhill and Pack[18] found the pituitrin affected kidney less sensitive than the normal kidney to the hydremic stimulus; hence pituitrin is not diuretic but relatively antidiuretic. They state further that renal ligature does not modify the production of hydremia by pituitrin and that as pituitrin inhibits water diuresis the antidiuretic action and the hydremia are probably due to some alteration

of capillary permeability. Momose[19] asserts that on pituitrin injection the lymph flow and the blood concentration decrease and that the urine output first decreases but after a few minutes increases. Fromherz[20] believes that pituitrin acts in two phases, antidiuretic and diuretic. The duration of each phase differs according to the individual, so that pituitrin appears at times antidiuretic and at other times diuretic.

The effect of pituitrin on lymph flow may shed further light on its antidiuretic and diuretic action. The drug used was *pituitrin* (Parke, Davis & Co.), the dosage 0.2 gram per kg. body weight. The methods of measuring the lymph flow and the urine output were as described before. To know the concentration of blood, the hemoglobin content was determined after Sahli's method. The blood was always obtained from clean-cut incisions in the marginal ear veins of dogs.

TABLE XV

Piebald bitch, 5.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (6.6 c.c. in 55 c.c. of water).

| TIME | LYMPH C.C. | URINE C.C. | HEMOGLOBIN | NOTICE |
|---|---|---|---|---|
| 12.48′–58′ | 3.2 | 0.8 | | |
| 58′–68′ | 3.6 | 0.6 | 53 | |
| 1. 8′–18′ | 3.6 | 0.6 | 53 | |
| 18′–28′ | 3.1 | 0.6 | 52 | |
| 28′–38′ | 3.9 | 1.0 | 52 | |
| 38′–48′ | 3.2 | 1.0 | 54 | |
| 48′–58′ | 3.4 | 1.0 | 54 | |
| 58′–68′ | 1.8 | 5.0 | 54 | 58′0″–58′30″ pituitrin |
| 2. 8′–18′ | 1.1 | 3.2 | | 1.1 c.c. intravenously |
| 18′–28′ | 1.7 | 1.2 | 52 | |
| 28′–38′ | 2.4 | 1.2 | | |
| 38′–48′ | 2.7 | 0.8 | 50 | |
| 48′–58′ | 2.3 | 0.8 | | |
| 58′–68′ | 2.3 | 1.0 | 52 | |
| 3. 8′–18′ | 2.6 | 1.6 | | |
| 18′–28′ | 2.9 | 1.0 | 52 | |
| 28′–38′ | 3.2 | 1.2 | | |
| 38′–48′ | 2.2 | 0.8 | 54 | |
| 48′–58′ | 2.7 | 1.0 | | |
| 58′–68′ | 2.7 | 1.2 | 54 | |
| 4. 8′–18′ | 1.7 | 1.4 | | |
| 18′–28′ | 2.1 | 1.0 | 54 | |

The results of the intravenous injection of pituitrin are given in Table XV. We see that the lymph flow and the blood concentration decrease but that the urine output increases. We cannot see the first antidiuretic period; this is probably because the catheter was retained in the bladder; if the catheter had been in the ureter (Momose[19]), the antidiuretic period would have been perceived.

The results of the subcutaneous injection of pituitrin are given in Table XVI.

The lymph flow and the blood concentration decrease, but there is no increase of urine output.

The results of infusion with Gum-Ringer solution are given in Table XVII. The Gum-Ringer solution contains 2 per cent gum arabic in Ringer's solution for the purpose of keeping it within the vessel as long as possible. In Table XVII the results from infusion of this solution alone were observed as the

TABLE XVI

Piebald bitch, 6.5 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (7.8 c.c. in 65 c.c. of water).

| TIME | LYMPH C.C. | URINE C.C. | HEMOGLOBIN | NOTICE |
|---|---|---|---|---|
| 1. 5′–15′ | 4.3 | 1.1 | | |
| 15′–25′ | 4.1 | 1.0 | 72 | |
| 25′–35′ | 3.6 | 1.2 | 73 | |
| 35′–45′ | 3.2 | 1.0 | | |
| 45′–55′ | 3.1 | 1.0 | 72 | |
| 55′–65′ | 2.1 | 1.0 | | 55′0″–55′45″ pituitrin |
| 2. 5′–15′ | 2.4 | 1.2 | 73 | 1.3 c.c. subcutaneously |
| 15′–25′ | 1.4 | 1.4 | 70 | |
| 25′–35′ | 0.7 | 1.2 | | |
| 35′–45′ | 0.8 | 1.4 | 66 | |
| 45′–55′ | 1.2 | 1.4 | 66 | |
| 55′–65′ | 3.7 | 1.6 | | |
| 3. 5′–15′ | 4.0 | 1.4 | 68 | |
| 15′–25′ | 3.2 | 1.3 | | |
| 25′–35′ | 3.8 | 1.4 | 71 | |
| 35′–45′ | 2.3 | 1.6 | 71 | |
| 45′–55′ | 2.4 | 1.2 | 70 | |
| 55′–65′ | 2.8 | 1.4 | 70 | |
| 4. 5′–15′ | 2.9 | 1.3 | 74 | |
| 15′–25′ | 2.5 | 1.4 | 73 | |
| 25′–35′ | 3.2 | 1.8 | 75 | |
| 35′–45′ | 3.2 | 2.0 | | |
| 45′–55′ | 2.6 | 1.8 | | |

TABLE XVII

Yellow bitch, 4.2 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (5.0 c.c. in 42 c.c. of water).

| TIME | LYMPH C.C. | URINE C.C. | HEMOGLOBIN | NOTICE |
|---|---|---|---|---|
| 1.58′–68′ | 3.4 | 0.4 | 50 | |
| 2. 8′–18′ | 2.8 | 0.8 | 50 | |
| 18′–28′ | 2.8 | 0.6 | 50 | |
| 28′–38′ | 2.8 | 0.4 | 50 | |
| 38′–48′ | 8.2 | 0.4 | | 38′–55′ Gum-Ringer solution |
| 48′–58′ | 10.5 | 1.0 | 47 | 63 c.c. intravenously |
| 58′–68′ | 7.4 | 0.6 | 45 | |
| 3. 8′–18′ | 4.0 | 0.4 | 47 | |
| 18′–28′ | 3.7 | 0.8 | 50 | |
| 28′–38′ | 3.7 | 0.6 | | |
| 38′–48′ | 2.1 | 1.0 | 50 | |
| 48′–58′ | 2.5 | 1.0 | | |
| 58′–68′ | 2.5 | 0.4 | 50 | |

control. This infusion causes a diminution in blood concentration and an increase in lymph flow but no change in urine output.

In Tables XVI to XVIII I would draw attention to the fact that while pituitrin alone subcutaneously, or Gum-Ringer solution alone intravenously, do not cause an increased urine output, their *simultaneous* administration, by the same routes, does produce an increase in urine flow. The blood concentration is more decreased, while the lymph flow increases much less than the Gum-Ringer control. Thus we see that the urine output increases after intravenous injection (Table XV), but does not after subcutaneous injection of pituitrin (Table XVI), while the lymph flow decreases in both cases. Therefore it is evident that the increase in urine output is not the cause of lymph

TABLE XVIII

Piebald bitch, 4.3 kg. Fasted 36 hours before the operation. Narcotized with paraldehyde (5.1 c.c. in 43 c.c. of water).

| TIME | LYMPH c.c. | URINE c.c. | HEMOGLOBIN | NOTICE |
|---|---|---|---|---|
| 3.58′–68′ | 2.8 | 1.0 | 52 | |
| 4. 8′–18′ | 2.7 | 1.0 | 52 | |
| 18′–28′ | 2.9 | 1.0 | 52 | |
| 28′–38′ | 2.3 | 1.0 | 52 | 37′0″–37′30″ pituitrin |
| 38′–48′ | 4.0 | 0.4 | | 0.86 c.c. subcuaneously |
| 48′–58′ | 6.3 | 3.4 | 46 | |
| 58′–68′ | 5.9 | 3.8 | 46 | 38′–53′ Gum-Ringer solution |
| 5. 8′–18′ | 5.7 | 2.6 | 48 | 64.5 c.c. intravenously |
| 18′–28′ | 3.8 | 1.6 | 50 | |
| 28′–38′ | 3.4 | 1.2 | 48 | |
| 38′–48′ | 3.3 | 0.8 | 47 | |
| 48′–58′ | 3.2 | 0.6 | 48 | |
| 58′–68′ | 3.5 | 0.6 | 51 | |
| 6. 8′–18′ | 3.2 | 0.6 | 52 | |
| 18′–28′ | 2.9 | 0.6 | | |
| 28′–38′ | 2.9 | 0.6 | 52 | |

diminution. Since, however, the intravenous injection of pituitrin causes an hydremic plethora and at the outset an antidiuresis, it is probable that pituitrin inhibits the secretory action of endothelial cells of the blood capillaries and of the kidney glomeruli. The diminution of lymph flow is due to inhibition of secretion by the capillary endothelium. Because of the inhibiting action on both glomeruli and blood capillaries, the hydremia will be more and more heightened after the injection of pituitrin; and when the hydremic stimulus overcomes the inhibiting action of pituitrin, the period of antidiuresis changes to that of diuresis. This is the reason why pituitrin is both antidiuretic and diuretic.

The stimulus of the hydremia caused by subcutaneous injection of pituitrin alone is not enough to overcome the inhibiting action, but if the Gum-Ringer solution is supplied at the same time and the hydremia thus increased, the inhibition no longer dominates. The insistence of Underhill and Pack[18] that on injection of pituitrin the increased urine output is very slight compared to the marked diminution of blood concentration, also explains the inhibiting action on the secretion of both blood capillaries and glomeruli.

## SUMMARY AND CONCLUSION

1. The second class of lymphagogues stimulates the secretory activity of endothelial cells of both blood capillaries and glomeruli. Since both are stimulated not only the lymph flow but also the urine output is markedly increased.

2. The first class of lymphagogues promotes the migration of tissue fluids into the lymphatic system. The urine output increases owing to the passage of the tissue fluids into the circulation through the lymphatics. It does not increase so much following the second class which stimulates secretion in the kidney.

3. Pituitrin inhibits the secretion of endothelial cells of both blood capillaries and glomeruli. Therefore the lymph flow, the blood concentration, and

the urine output decrease; but the urine output increases soon after the stimulus of the hydremia is heightened.

It is probable that even in the normal state such substances, which act like the lymphagogues, appear in the lymph spaces as products of metabolism. Some of them increase the tissue fluids, attracting water from the tissue cells and the blood, and others promote the migration of the tissue fluids into the lymphatics. Accordingly, the lymph flowing out of the thoracic duct may contain the excretions produced by the metabolism of tissue cells. To this extent, the cellular physiologic theory may be true, but that the lymphagogues themselves increase the functions of organs, is a theory with objectionable points.

Thus the lymphatic system belongs to the excreting system and it plays a great part in regulating the constitution of the blood. An abnormal, either qualitative or quantitative substance, existing in the blood, must be excreted on one hand by the kidney and on the other by the blood capillaries into the lymph spaces where part of it will be fixed by the tissue cells and part goes farther into the lymphatics. It returns again into the circulation through the lymphatic system to be excreted finally through the kidney.

Thus the abnormal substance will be in part conserved in the lymphatic system and in the tissue cells for a while, in order that the kidney will not be burdened too much at one time. In this sense we may call the tissues the "forekidney."

The blood capillaries act to the same purpose as the glomeruli, the latter carrying the catabolic substance out of the body, the former sending it into the lymph spaces. From this statement the blood capillaries may be called "pre-glomeruli."

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# THE ANTIKETOGENIC INFLUENCE OF INSULIN IN DIABETES MELLITUS*

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BANTING, Campbell, and Fletcher[1] have observed the disappearance of ketone bodies from the urine of diabetic patients four hours after the administration of insulin and their reappearance in eight hours. After repeated large doses of insulin Campbell[2] found a decrease of the ketone bodies of the blood and urine with an increase of the carbon dioxide combining power of the blood plasma and an increase in the tension of carbon dioxide of the alveolar air. In these data, however, the level of the carbon dioxide combining power is not always dependent upon changes in the concentration of the ketone bodies of the blood or urine. Rabinowitch[3] obtained a fall of the blood ketone bodies and a rise in the carbon dioxide combining power after insulin, but he concludes that the rise in the carbon dioxide combining power is not proportional to the drop of blood ketone bodies. These reports demonstrate the efficacy of insulin therapy in diabetes; however, their value in demonstrating its antiketogenic action is open to question, since other well-known antiketogenic agents such as glucose, saline solutions, and alkalies were utilized to relieve the acidosis.

The object of the present study was to determine the immediate action of insulin upon the production and excretion of the ketone bodies and upon the acid base equilibrium of the blood in human cases of diabetes mellitus. In order to eliminate the dietetic factor the subjects were starved for twelve to fourteen hours previous to the administration of the insulin. Also, during the period of observation (from eight to ten hours), food was withheld, but the drinking of water was permitted *ad libitum*. As a control on the effect of the starvation itself upon the blood, the blood sugar and carbon dioxide combining power were determined in five cases at two hourly intervals for eight to ten hours under similar conditions of food privation and both with and without insulin. Starvation without insulin continuously decreased the blood sugar. The lowest level reached was about 71 per cent of the original concentration. The drop in blood sugar after the insulin was, however, much greater, a level of 50 to 30 per cent of the original concentration was obtained. This decrease reached its maximum in four to six hours and was followed by an appreciable rise. In 3 instances the carbon dioxide combining power rose steadily during starvation, but in the 2 remaining cases a continuous fall was noted. On the contrary, after the insulin in all of the five cases a rise in the carbon dioxide combining power was noted for six hours with subsequently a slight drop. Evidently the restriction of food was a negligible quantity in influencing the results to be discussed.

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Since it was planned to study the antiketogenic effect of insulin alone, it was essential to avoid the development of hypoglycemic shock necessitating the use of emergency measures. In an extensive series of preliminary experiments it was found that one unit of insulin would decrease the blood sugar of an individual weighing from 50 to 70 kg. about 0.008 per cent in four to six hours. Upon the basis of this observation, the dose of insulin was graded to obtain the maximum beneficial effect without producing untoward results. The units of insulin employed in the following experiments were the quantities calculated to reduce the blood sugar to about 0.150 per cent, and it was the only therapeutic agent utilized. A control specimen of blood was obtained before and after the injection of the insulin, at one- or two-hour intervals, until its influence upon the sugar and carbon dioxide combining power had vanished. Specimens of urine were also collected at corresponding intervals and preserved with toluene until analyzed. The control specimen of urine represented the collection over a one- or two-hour period before the insulin. The insulin was injected subcutaneously. The specimens of blood were analyzed immediately after withdrawal by venipuncture to avoid any source of error due to glycolysis or to production of fixed acid bodies in the blood upon standing. The blood sugar was determined by the Folin-Wu technic and the carbon dioxide combining power by the Van Slyke method. Van Slyke and Fitz's[4] gravimetric method was employed for the ketone bodies of the blood, and the procedure described by Van Slyke[5] was used for the urine. In the blood the total ketone bodies were calculated as acetone in milligrams per 100 c.c., and in the urine the total ketone bodies are reported as acetone in concentration per cent, and as grams excreted during the interval. The urine sugar was estimated by Benedict's most recent method,[6] and where the amount exceeded 0.5 per cent, parallel determinations were made with the same author's volumetric method.[7]

## DISCUSSION OF RESULTS

In Table I are presented the results obtained in 10 series of observations upon 7 cases of diabetes. In the first 2 of these, analyses were confined to the blood, since it was impossible to obtain samples of urine at the proper intervals from these patients who at the outset were in coma. It will be noted that in all instances there is a prompt decrease in the blood sugar reaching a minimum concentration of about 50 per cent of the control figure. The lowest value obtained was 0.096 in Case 10. The control specimen of blood from this patient showed a pronounced lipemia and since it was desired to observe the influence of the insulin on the blood lipoids, an unusually large dose was administered in this case.* In no instance was a hypoglycemia produced. It is worthy of note that in but five of the ten cases was the calculated effect of the insulin upon the sugar obtained.

More striking, however, are the changes observed in the urine sugar. During the control periods the urines contained from 1.06 to 7.8 gm. of sugar, but after the insulin administration it was rapidly reduced to well within nor-

*A more complete report of this case has been made by Lough and Killian, Medical Clinics of North America, July, 1924, viii, 337.

TABLE I

CHEMICAL CHANGES IN BLOOD AND URINE AFTER INSULIN

| CASE | TIME | BLOOD | | | URINE | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SUGAR PER CENT | $CO_2$ C. P. VOL. PER CENT | KETONE BODIES MG. PER 100 C.C. | VOL. C.C. | SUGAR | | KETONE BODIES | | |
| | | | | | | PER CENT | GM. | PER CENT | GM. | |
| 1—L. C. | 2.30 P.M. | 0.514 | 12.6 | 87.8 | | | | | | Blood plasma PH = 7.13 |
| Age = 28—M. | 3.30 " | Insulin | 40 units | | | | | | | |
| 6/5/23 | 5.30 " | 0.312 | 26.3 | 60.4 | | | | | | |
| | 7.30 " | 0.240 | 32.8 | 44.8 | | | | | | |
| | 9.30 " | 0.300 | 39.3 | 29.8 | | | | | | |
| | 11.30 " | 0.308 | 38.4 | 29.8 | | | | | | |
| | 12.00 " | Insulin | 15 units | | | | | | | |
| 6/6/23 | 8.15 A.M. | 0.254 | 37.4 | 27.5 | | | | | | Blood plasma PH = 7.28 |
| 2—A. W. | 10.55 P.M. | 0.468 | 20.4 | 54 | | | | | | |
| Age = 39—F. | 11.00 " | Insulin | 20 units | | | | | | | |
| 7/31/23 | 1.00 A.M. | 0.364 | 22.1 | 39.8 | | | | | | |
| 8/ 1/23 | 3.00 " | 0.332 | 25.8 | 30.6 | | | | | | |
| | 5.00 " | 0.384 | 28.1 | 25.2 | | | | | | |
| | 6.00 " | Insulin | 20 units | | | | | | | |
| | 1.00 P.M. | 0.306 | 34.3 | 15.0 | | | | | | |
| 3—N. L. | 8.20A.M. | 0.308 | 32.8 | | 197 | 2.50 | 4.92 | 0.62 | 1.22 | Urine collected 8.20-9.30 A.M. |
| Age = 36—M. | 9.30 " | Insulin | 20 units | | | | | | | |
| 1/6/23 | 10.30 " | 0.262 | 34.7 | | 100 | 2.63 | 2.63 | 0.45 | 0.45 | |
| | 11.30 " | 0.222 | 41.4 | | 75 | 1.0 | 0.75 | 0.24 | 0.18 | |
| | 12.30 P.M. | 0.178 | 42.4 | | 34 | 0.74 | 0.25 | 0.15 | 0.052 | |
| | 1.30 " | 0.158 | 46.2 | | 110 | 0.02 | 0.022 | 0.055 | 0.061 | |
| | 2.30 " | 0.151 | 44.3 | | 125 | 0.05 | 0.062 | 0.044 | 0.055 | |
| | 3.30 " | 0.172 | 44.3 | | | | | | | |
| | 4.30 " | 0.192 | 41.4 | | 163 | 0.03 | 0.048 | 0.051 | 0.084 | |

TABLE I—CONT'D

| CASE | TIME | BLOOD | | | URINE | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SUGAR PER CENT | $CO_2$ C. P. VOL. PER CENT | KETONE BODIES MG. PER 100 C.C. | VOL. C.C. | SUGAR | | KETONE BODIES | | |
| | | | | | | PER CENT | GM. | PER CENT | GM. | |
| 4—L. C. | 9.00 A.M. | 0.342 | 30.9 | | 250 | 0.80 | 2.0 | 0.76 | 1.90 | Urine collected 7.15-9.15 A.M. |
| Age = 28—M. | 9.15 " | Insulin | 30 units | | | | | | | |
| 3/9/23 | 11.15 " | 0.197 | 35.7 | | 29 | 0.46 | 0.13 | 0.65 | 0.19 | |
| | 1.15 P.M. | 0.180 | 38.5 | | 66 | 0.01 | 0.006 | 0.40 | 0.266 | |
| | 3.15 " | 0.131 | 40.4 | | 65 | 0.01 | 0.006 | 0.15 | 0.104 | |
| | 5.15 " | 0.104 | 44.3 | | 42 | 0.01 | 0.004 | 0.14 | 0.060 | |
| 3/10/23 | 12.30 A.M. | 0.255 | 36.6 | | | | | | | |
| 5—F. L. | 8.55 A.M. | 0.226 | 35.3 | | 180 | 3.2 | 5.76 | 0.41 | 0.748 | Urine collected 7.00-9.00 A.M. |
| Age = 34—M. | 9.00 " | Insulin | 11 units | | | | | | | |
| 7/23/23 | 11.00 " | 0.226 | 38.1 | | 75 | 3.0 | 2.25 | 0.22 | 0.174 | |
| | 1.00 P.M. | 0.168 | 33.4 | | 345 | 0.34 | 1.20 | 0.042 | 0.148 | |
| | 3.00 " | 0.178 | 33.4 | | 35 | 2.2 | 0.77 | 0.19 | 0.067 | |
| | 5.00 " | 0.254 | 32.4 | | 105 | 2.1 | 2.20 | 0.22 | 0.231 | |
| 6—A. Y. | 10.00 A.M. | 0.302 | 36.6 | | 210 | 3.5 | 7.35 | 0.36 | 0.760 | Urine collected 9.10-10.10 A.M. |
| Age = 20—M. | 10.10 " | Insulin | 22 units | | | | | | | |
| 1/10/23 | 11.10 " | 0.274 | 40.4 | | 65 | 3.0 | 1.95 | 0.45 | 0.294 | |
| | 12.10 P.M. | 0.220 | 52.8 | | 30 | 1.5 | 0.45 | 0.33 | 0.100 | |
| | 1.10 " | | | | 305 | 0.01 | 0.03 | 0.018 | 0.055 | |
| | 2.10 " | 0.184 | 56.7 | | 255 | 0.01 | 0.02 | 0.011 | 0.029 | |
| | 3.15 " | 0.193 | 51.9 | | 45 | 0.11 | 0.04 | 0.18 | 0.081 | |
| | 4.15 " | 0.157 | 51.0 | | 115 | 0.08 | 0.09 | 0.054 | 0.063 | |
| | 5.10 " | 0.190 | 51.0 | | 35 | 0.19 | 0.09 | 0.28 | 0.098 | |

TABLE I—CONT'D

| CASE | TIME | BLOOD | | | URINE | | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SUGAR PER CENT | $CO_2$ C. P. VOL. PER CENT | KETONE BODIES MG. PER 100 C.C. | VOL. C.C. | SUGAR PER CENT | SUGAR GM. | KETONE BODIES PER CENT | KETONE BODIES GM. | |
| 7—S. G. | 4.00 P.M. | 0.288 | 22.3 | 60.3 | 285 | 2.5 | 7.12 | 1.07 | 3.05 | Urine collected 3.30-5.30 P.M. |
| Age = 22—M. | 5.30 " | Insulin | 22 units | | | | | | | Blood plasma PH = 7.24 |
| 3/29/23 | 7.30 " | 0.205 | 23.3 | 51.2 | 265 | 1.4 | 3.65 | 0.472 | 1.25 | |
| | 9.30 " | 0.182 | 28.1 | 46.0 | 195 | 0.18 | 0.35 | 0.115 | 0.224 | Blood plasma PH = 7.34 |
| | 11.30 " | 0.151 | 32.8 | 43.9 | | | | | | |
| 3/30/23 | 1.30 A.M. | 0.176 | 30.0 | 45.5 | 120 | 0.18 | 0.22 | 0.13 | 0.156 | |
| | 8.30 " | 0.267 | 30.0 | 53.4 | 225 | 0.40 | 0.90 | 0.50 | 1.125 | |
| 8—A. W. | 10.00 A.M. | 0.282 | 24.9 | 45.3 | 230 | 2.3 | 5.29 | 0.32 | 0.743 | Urine collected 8.20-10.20 A.M. |
| Age = 51—M. | 10.20 " | Insulin | 20 units | | | | | | | |
| 7/6/23 | 12.20 P.M. | 0.280 | 26.8 | 45.6 | 225 | 2.8 | 6.30 | 0.29 | 0.657 | |
| | 2.20 " | 0.254 | 29.6 | 30.2 | | | | | | |
| | 4.20 " | 0.274 | 26.8 | 26.6 | 185 | 2.3 | 4.25 | 0.14 | 0.259 | |
| | 5.00 " | Insulin | 20 units | | | | | | | |
| | 6.20 " | 0.264 | 32.4 | 19.6 | 175 | 2.1 | 3.67 | 0.097 | 0.171 | |
| 9—N. L. | 9.00 A.M. | 0.308 | 46.2 | 32.9 | 88 | 1.7 | 1.47 | 0.30 | 0.260 | Urine collected 9.00-10.00 A.M. |
| Age = 36—M. | 10.00 " | Insulin | 23 units | | | | | | | |
| 1/12/23 | 11.05 " | 0.256 | 51.9 | 32.0 | 56 | 1.1 | 0.61 | 0.18 | 0.101 | " " 10.30-11.00 " |
| | 12.05 P.M. | 0.220 | 57.6 | 13.0 | 24 | 0.28 | 0.067 | 0.066 | 0.016 | " " 11.30-12.30 P.M. |
| | 1.06 " | 0.180 | 60.5 | 2.8 | 222 | 0.02 | 0.044 | 0.014 | 0.033 | " " 12.30- 1.30 " |
| | 2.12 " | 0.150 | 59.5 | 14.0 | 470 | 0.01 | 0.047 | 0.004 | 0.020 | " " 1.30- 2.30 " |
| | 3.05 " | 0.172 | 59.5 | 14.0 | 91 | 0.04 | 0.036 | 0.030 | 0.028 | " " 2.30- 3.30 " |
| | 4.08 " | 0.190 | 57.6 | 15.1 | 140 | 0.02 | 0.028 | 0.027 | 0.038 | " " 3.30- 4.30 " |
| | 5.08 " | 0.181 | 54.8 | 17.0 | 150 | 0.02 | 0.030 | 0.027 | 0.041 | " " 4.30- 5.28 " |
| 10—A. Y. | 3.30 P.M. | 0.300 | 41.8 | 31.0 | 260 | 3.0 | 7.80 | 0.26 | 0.681 | Urine collected 3.00-5.00 P.M. |
| Age = 21—M. | 5.00 " | Insulin | 35 units | | | | | | | |
| 12/15/23 | 7.00 " | 0.134 | 42.8 | 29.6 | 360 | 0.55 | 1.98 | 0.14 | 0.504 | |
| | 9.00 " | 0.122 | 49.4 | 20.9 | 150 | 0.17 | 0.25 | 0.19 | 0.288 | |
| | 10.00 " | 0.096 | 53.2 | 16.0 | 210 | 0.04 | 0.084 | 0.03 | 0.063 | |

mal limits. The lowest level reached by the urine sugar in each case was about 0.5 per cent of the control figure and is synchronous with the minimum concentration of the blood sugar.

The study of changes in the ketone bodies of the blood and urine in conjunction with the alkali reserve of the blood plasma after insulin reveals a very significant effect of the substance. In the first two cases the administration of insulin resulted in a continuous decrease of the blood ketone bodies with a steady rise in the carbon dioxide combining power of the plasma. Van Slyke and Fitz have placed the normal concentration of total ketone bodies as acetone between 1.3 and 2.6 mg. per 100 c.c. Subject 1 showed a drop in the ketone bodies of the blood from 87.8 mg. to 29.8 mg. per 100 c.c. during the six hours following a dose of 40 units of insulin. During the same period the carbon dioxide capacity of the blood plasma rose from 12.6 to 39.3 volumes per cent. Eight hours after a second dose of 15 units, the ketone bodies had fallen to 27.5 mg., and the carbon dioxide combining power rose to 37.4 volumes per cent. It is worthy of note that the minimum concentration of ketone bodies corresponds in time with the maximum carbon dioxide capacity of the blood plasma. A similar effect is observed in Case 2 in which the ketone bodies are diminished from 54 to 25.2 mg., paralleled by a rise in the carbon dioxide combining power of the blood plasma from 20.4 to 28.1 volumes per cent in six hours. Here again a second dose of 15 units brought the carbon dioxide capacity up to 34.3 volumes per cent, and the ketone bodies were decreased to 15 mg. per 100 c.c.

In the following 4 cases the excretion of the ketone bodies of the urine has been studied in association with the sugar and carbon dioxide combining power of the blood. All of the data indicate a prompt and continuous decrease in the ketonuria after insulin. It is evident that although the concentration of these compounds in the urine varies with the volume of urine, the actual output is independent of the volume excreted. There is no evidence of the initial rise and the subsequent fall in the ketonuria, noted by Mosenthal and Killian,[8] after sodium bicarbonate administration. The maximum excretion of ketone bodies in the urine of normal men under usual conditions reported by Van Slyke is about 0.42 gm. per liter calculated as acetone, i.e., 0.042 per cent. All of the subjects reported in the table showed markedly increased concentration of urine ketone bodies during the control period, from 0.75 to 1.90 gm., i.e., 0.41 to 0.76 per cent. The average decrease was to 5 to 10 per cent of the control figure, to 0.067 to 0.029 gm. (0.19 to 0.011 per cent). This minimum concentration of urinary ketone bodies is encountered at about the time the carbon dioxide combining power has reached its maximum. Subject 5, however, shows a lag in the decrease of the ketonuria and hyperglycemia. Here the carbon dioxide capacity rose but 2.8 volumes per cent and that during the first two hours. In this instance the diabetes was complicated by other pathologic conditions.

In the final cases, from 7 to 10 inclusive, concurrent determinations of both the ketone bodies of the blood and urine have been made. The control specimens of blood showed ketone bodies varying from 31 to 60.3 mg. per 100 c.c., whereas the initial urine specimens contained from 0.267 to 3.05 gm.

of these compounds. Following the administration of the insulin there is a continuous decrease in the blood ketone bodies; this drop parallels the rise in the carbon dioxide combining power of the plasma. In Case 9 an unusual fall of the blood ketones from 32.9 to 2.8 mg. per 100 c.c. was noted, but in the other instances the average decrease is but 50 per cent of the initial concentration. A simultaneous but far more striking decrease of the ketone bodies of the urine is seen, the average reaching 10 to 30 per cent of the initial figure. As observed above for the urine sugar, the effect of the insulin upon the ketone bodies of the urine is more pronounced than upon the ketone bodies of the blood. It is of interest to note that in Subjects 1, 2, and 8 the influence of the insulin upon the ketosis is magnified by the administration of a second dose after the maximum effect of the first dose has been obtained. In the remaining cases, a rise of the sugar and ketone bodies of the blood and urine and a decrease of the carbon dioxide combining power of the blood plasma appear six to eight hours after the insulin. The rise of the ketone bodies, however, follows the increase of the sugar.

It is evident from the data presented that the decrease of the ketone bodies, both of the blood and urine, is accompanied by a rise in the alkali reserve of the blood plasma. On the basis of the statement of Palmer and Van Slyke[9] that 0.5 gm. of sodium bicarbonate will raise the carbon dioxide combining power one volume per cent for each forty-two pounds of body weight, it has been calculated from the results recorded that one unit of insulin is equivalent in its influence upon the alkali reserve of the blood to about 0.7 gm. of sodium bicarbonate taken orally. Such a comparison is, however, but a rough approximation, since all of the subjects studied manifested diabetic ketosis. In such conditions, as Palmer and Van Slyke state, the organism is forming acid at a rapid rate; hence the sodium bicarbonate will elevate the plasma bicarbonate by less than the calculated amount because part of the alkali given is neutralized by the acids formed during the necessarily gradual administration.

Determinations of the plasma $P_H$ were made by the method of Myers, Schmitz, and Booher[10] in two subjects (Cases 1 and 7). In the first instance the plasma $P_H$ was 7.13 with a plasma $CO_2$ capacity of 12.6 volumes per cent. After the administration of 55 units of insulin in two doses, the $CO_2$ capacity of the plasma had reached 37.4 volumes per cent with a $P_H$ of 7.28. Again in Case 7 the control blood plasma showed a $CO_2$ capacity of 22.3 per cent and a $P_H$ of 7.24. Four hours later the $CO_2$ capacity had risen to 28.1 and the $P_H$ to 7.34. These findings indicate that insulin induces a return towards normal of both the carbon dioxide combining power and of the $P_H$ of the plasma. These observations are in keeping with the statement of Cullen and Jonas[11] that under insulin treatment the alkali reserve and the $P_H$ of the blood plasma return to their normal level coincidently, and there appears to be a consistent relation between these two factors during their return to normal.

It is apparent that the improvement in the alkali reserve of the blood plasma is dependent upon the inhibition of the ketogenesis. Corresponding to the decrease in the ketosis there is noted a drop in the hyperglycemia and glycosuria. Ringer[12] has reported in phlorizinized dogs a rise in the respiratory

quotient after insulin and an enhanced ability to metabolize glucose. This author found that in the experimental animals one unit of insulin would effect the complete oxidation of 2 gm. of glucose per hour. Briggs, Koechig, Doisy, and Weber[13] have shown that under the influence of insulin the liver of phlorizinized dogs regains the ability to convert glucose into lactic acid and subsequently to dispose of this by oxidation. Dudley and Marrian[14] report that the disappearance of sugar from the blood after insulin cannot be attributed to an increased storage of glycogen in liver or muscles. Lyman, Nicholls, and McCann[15] have demonstrated, moreover, a rise in the respiratory quotients and of the heat production in normal human subjects after insulin; hence it is logical to attribute the diminution of the hyperglycemia and the glycosuria of diabetes to an increased oxidation of glucose stimulated by the insulin. A more complete oxidation of the fatty acids would thus be made possible, resulting in a diminution of the ketone bodies. The antiketogenic influence of insulin in diabetes presumably is due to this sequence of events.

## SUMMARY

1. The administration of insulin to cases of diabetes mellitus results in an immediate decrease of the ketone bodies of the blood and urine, reaching the maximum in four to six hours.

2. The decrease in the ketosis is accompanied by a corresponding rise in the alkali reserve of the blood plasma.

3. The rise in the carbon dioxide capacity is associated with a proportional increase in the blood plasma $P_H$.

4. The antiketogenic influence of the insulin apparently is due to the more complete oxidation of the carbohydrates stimulated by this substance.

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# GALACTOSE TOLERANCE IN LATENT TETANY*

By Max Erdheim, M.D., Brooklyn, N. Y.

DISTURBANCES of carbohydrate metabolism are almost constant in dogs in which tetany has been induced by the removal of the parathyroid glands. According to Falta,[1] the assimilation limits for dextrose in parathyroidectomized dogs are regularly reduced. Eppinger, Falta, and Rudinger[2] observed a lasting glycosuria in a completely parathyroidectomized dog sustained on a diet not very rich in starchy material. Falta and Rudinger[3] found that the injection of adrenalin into such animals brought about a marked glycosuria. In this connection one may mention the alimentary glycosuria observed in thyroparathyroidectomized dogs by Falkenberg[4] and by Underhill and Tadasu-Saiki;[5] these authors, however, considered this phenomenon to be the result of a hypofunction of the thyroid gland. In brief, many observations are in accord to the effect that parathyroidectomy in dogs produces, in association with the artificial tetany, definite and constant abnormalities of carbohydrate metabolism.

In cases of human tetany, disturbances of carbohydrate metabolism similar to those in parathyroidectomized dogs are not commonly observed. Falta and Kahn[6] could not demonstrate such a corresponding decreased tolerance for dextrose in human cases. Only in one case, an acute attack of tetany complicating Graves' disease, did they observe alimentary glycosuria after the acute phase had passed; but they were unable to provoke it again, although they made several attempts with that end in view. Even in this case the objection might well be raised that they were really dealing with a period of glycosuria accompanying Graves' disease, for this complication is rather common in that condition. In view of the striking changes in the carbohydrate metabolism observed in dogs with artificial tetany, it seemed advisable to investigate more fully human cases with reference to the possible existence of similar conditions. If such changes could likewise be demonstrated in human cases, it is obvious that a knowledge of them would be of definite clinical value, since it would furnish the basis for a convenient laboratory test to aid in the recognition of latent tetany.

In this investigation galactose was the sugar chosen for the tolerance tests. The average tolerance for this carbohydrate has been carefully worked out. Rowe[7] demonstrated that the tolerance dose of galactose is 30 gm. for men and 40 gm. for women. The usual amount of galactose given in my tests was 40 gm., although this was not always the case. The dosage of galactose per kilogram of body weight was computed, and these figures are expressed in the tabulation. The blood-sugar level was estimated before the intake of the galactose and at half-hour intervals thereafter until its return to the pre-

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TABLE I

GALACTOSE TOLERANCE IN FIVE CASES OF OUTSPOKEN TETANY

| CASE | SEX | AGE | WEIGHT IN KG. | GM. OF GALACTOSE BY MOUTH | | BEFORE GALACTOSE INTAKE | PERCENTAGE OF BLOOD SUGAR AFTER GALACTOSE INTAKE | | | | | GM. SUGAR IN URINE | ELECTRICAL TESTS MILLIAMPERES | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ABSOLUTE | PER KG. BODY WEIGHT | | ½ H. | 1 H. | 1½ H. | 2 H. | 2½ H. | | KCC | ACC | AOC |
| 1 | M | 18 | 37.0 | 40.0 | 1.08 | 0.12 | 0.15 | 0.11 | 0.11 | 0.11 | | 2.50 | 0.6 | 1.4 | 1.0 |
| 2 | M | 21 | 60.9 | 40.0 | 0.65 | 0.11 | 0.15 | 0.14 | 0.12 | 0.10 | 0.10 | 0.40 | 0.8 | 1.9 | 1.8 |
| 3 | M | 20 | 50.0 | 34.0 | 0.68 | 0.12 | 0.14 | 0.14 | 0.11 | 0.10 | | 0.15 | 0.9 | 1.9 | 2.0 |
| 4 | M | 50 | 59.5 | 40.5 | 0.68 | 0.10 | 0.13 | 0.16 | 0.11 | 0.10 | 0.10 | 1.80 | | | |
| 5 | M | 18 | 48.0 | 32.6 | 0.68 | 0.11 | 0.14 | 0.11 | 0.10 | 0.11 | | 0.80 | 0.7 | 1.6 | 1.5 |
| Average | | 25 | 51.0 | 37.4 | 0.75 | 0.11 | 0.14 | 0.13 | 0.11 | 0.10 | 0.10 | 1.13 | | | |

TABLE II

GALACTOSE TOLERANCE IN SEVEN CONTROL CASES

| CASE | SEX | AGE | WEIGHT IN KG. | GM. GALACTOSE BY MOUTH PER KG. BODY WEIGHT* | BEFORE GALACTOSE INTAKE | PERCENTAGE OF BLOOD SUGAR AFTER GALACTOSE INTAKE | | | | | | GM. SUGAR IN URINE | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ½ H. | 1 H. | 1½ H. | 2 H. | 2½ H. | 3 H. | | |
| 1 | F | 26 | 59.0 | 0.68 | 0.12 | 0.17 | 0.15 | 0.11 | 0.11 | 0.11 | 0.12 | 0.20 | traumatic neurosis |
| 2 | M | 17 | 56.3 | 0.71 | 0.11 | 0.15 | 0.19 | 0.14 | 0.11 | 0.11 | 0.11 | 2.26 | chronic polyarthritis |
| 3 | F | 34 | 64.7 | 0.62 | 0.11 | 0.16 | 0.16 | 0.12 | 0.10 | 0.12 | 0.10 | 0.85 | stomach catarrh (cured) |
| 4 | M | 31 | 63.0 | 0.63 | 0.11 | 0.17 | 0.15 | 0.11 | 0.09 | 0.09 | 0.09 | 0.46 | pericarditis (cured) |
| 5 | F | 56 | 50.0 | 0.80 | 0.11 | 0.12 | 0.16 | 0.12 | 0.10 | 0.09 | 0.09 | 0.30 | normal |
| 6 | F | 39 | 55.0 | 0.73 | 0.12 | 0.16 | 0.17 | 0.12 | 0.11 | 0.11 | 0.11 | 1.20 | pulmonary t. b. (inactive) |
| 7 | F | 39 | 65.0 | 0.62 | 0.11 | 0.16 | 0.13 | 0.11 | | | | 0.70 | hysteria |
| Average | | 35 | 59.0 | 0.68 | 0.11 | 0.16 | 0.16 | 0.12 | 0.10 | 0.10 | 0.10 | 0.85 | |

*Forty grams of galactose given to each subject.

alimentary value. The blood-sugar determinations were made by means of Bang's method.

Five patients with outspoken symptoms of tetany were studied. All of these individuals exhibited Trousseau's sign and Chvostek's phenomenon, and their electrical reactions were of the Erb type. The data with reference to these subjects are given in Table I.

Seven individuals who were either normal or had conditions that could have no possible effect upon the galactose tolerance were utilized as controls. The data with reference to the controls are given in Table II.

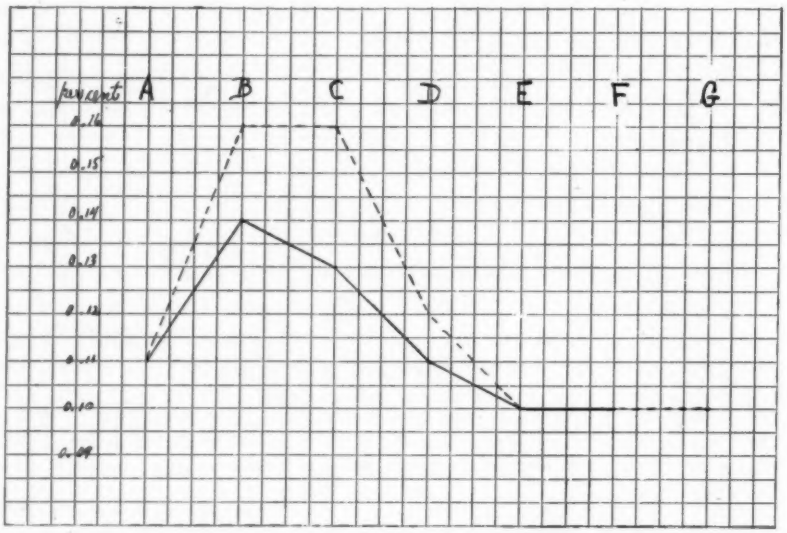


Fig. 1.—Blood-sugar curves after ingestion of approximately 40 gm. of galactose. Solid line, composite curve of five cases of outspoken tetany; interrupted line, composite curve of seven control cases. *A*. before galactose intake; *B*. one-half hour after; *C*. one hour after; *D*. one and a half hours after; *E*. two hours after; *F*. two and a half hours after; *G*. three hours after. Sugar determinations by Bang's method.

The results of this study are shown in Tables I and II and are graphically represented in the accompanying figure. Both the tables and the composite graph (Fig. 1) show that in the 5 cases of human tetany studied there was no decrease in the galactose tolerance; on the contrary, the tolerance was somewhat increased. Thus the changes in the carbohydrate metabolism observed in parathyroidectomized dogs cannot be duplicated in human tetany, and the study of the galactose tolerance does not offer a means of recognizing latent tetany.

## SUMMARY

Five patients with outspoken symptoms of tetany and 7 control subjects were studied by means of blood-sugar curves as to their sugar tolerance after the ingestion of approximately 40 gm. of galactose. It was thought that the constant changes in the carbohydrate metabolism observed in parathyroidectomized dogs might possibly be duplicated in human cases of tetany. No decrease, however, in the galactose tolerance was found in these cases; on the contrary, the tolerance to this sugar was relatively somewhat increased. The

study of the galactose tolerance, therefore, does not offer a means of recognizing latent tetany in man.

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## THE INFLUENCE OF L. ACIDOPHILUS ON THE COLON-AEROGENES GROUP IN THE INTESTINE*

By Nicholas Kopeloff, Ph.D., and Philip Beerman, New York City

THE transformation or simplification of the human intestinal flora by means of L. acidophilus has been considered one of its salient features. In fact this phenomenon has been generally regarded as responsible for the advantages of L. acidophilus therapy. A review of the literature[1] leads one to the conclusion that in the successful treatment of constipation and diarrhea by L. acidophilus there is a relative increase in the number of aciduric organisms, compared with the number of proteolytic bacteria. The natural presumption is that such an increase is actual as well as relative. Yet there has been no experimental data offered in substantiation. The reduction in actual numbers of the colon-aerogenes group in the intestine is of importance not only in evaluating L. acidophilus therapy but in understanding its nature. The present investigation, therefore, was undertaken to throw some light on this problem.

A series of 36 epileptic male patients in the Manhattan State Hospital were subdivided into 3 groups for observation and treatment. Careful daily records were kept with regard to the number of convulsions, as well as the number of defecations, noting amount, color and consistency. These epileptics received the routine hospital diet. No selection of patients for the 3 groups was made other than to have an approximately equal distribution of young and old epileptics. For one month prior to any treatment, during treatment, and for two months subsequently, daily records were kept and weekly fecal analyses made. The methods used have been described elsewhere.[1] In addition to the microscopic examination of gram-stained smears made by our

*Read at the Society of American Bacteriologists, Dec. 30, 1925, at Madison, Wis. From the Bacteriology Department of the Psychiatric Institute, Ward's Island, N. Y. Received for publication, January 10, 1926.

modified method[1] and plated on whey agar, a special synthetic medium for the enumeration of organisms of the colon-aerogenes group[2] was employed in the two highest dilutions. A count of actual numbers was made by calculation of samples on the basis of dry weight.

Before considering the influence of L. acidophilus treatment it is of interest to discuss the results of feeding kaolin to epileptics. After the one month of preliminary observation 12 epileptics received 60 gm. of kaolin daily for about two months, 120 gm. of kaolin for the next ten days, and 240 gm. of kaolin for the final three weeks. Bacteriologists have always sought for the etiologic factor in epilepsy in the intestinal tract. If, as has been assumed, toxins are liberated in the intestinal tract of epileptics, then it seemed likely that kaolin, which has great absorbing capacity, would be of value. In a thought-provoking paper by Braafladt[3] it was claimed that kaolin "combines with toxins and toxic products of Vibro cholerae, B. dysenteriae (Shiga), B. enteritidis, B. diphtheriae, B. botulinus, B. typhosus, B. paratyphosus B, and perhaps of putrefactive and proteolytic bacteria, rendering them harmless. When taken by mouth over a period of from ten to thirty days in sufficient quantities (1 to 2 ounces per day for man), it changes the intestinal flora of rats, dogs, and men from a predominantly proteolytic type to an aciduric one."

According to Bergeim,[4] however, kaolin at first decreases and then increases intestinal reduction.

Briefly stated, kaolin had little if any influence in reducing the number of convulsions. Six of the 13 patients had more convulsions during treatments, 4 had approximately the same number, while 2 showed some diminution. The latter were within experimental error as shown by subsequent observations.

Kaolin did have a tendency to increase the number of normal daily defecations in 8 of the 12 patients. This was to be expected because of the improvement in physical character of the feces, which also became lighter in color. It is important, however, to note that there was no transformation from proteolytic to aciduric flora as was anticipated. The relative percentages of this group as calculated in gram-stained smears remained practically constant. On whey agar plates, with two exceptions, no L. acidophilus colonies were found; nor could it be concluded from the colon-aerogenes medium that a reduction in this group occurred.

There were 24 epileptics scheduled to receive L. acidophilus milk; these were divided into two groups. The first group comprised 12 patients, who, after a preliminary month of observation, received 1000 c.c. of sterile milk daily for more than a month. This served as a control for the subsequent ingestion of 1000 c.c. of L. acidophilus milk for a period of two months. Lactose was administered for the first two weeks of the period of L. acidophilus treatment to facilitate a transformation of the intestinal flora. The second group comprised 12 patients, who, after a preliminary month of observation, received 1000 c.c. of L. acidophilus milk for eleven days. Treatment was discontinued because of laboratory difficulties on all but 3 of these patients who were transferred to the first group later.

Considering the first group, the administration of 1000 c.c. sterile milk daily can be said to have had little if any influence on either the number of convulsions or the number of normal defecations. As was to be expected,[1] an increase in the number of normal defecations occurred when the same patients received 1000 c.c. of L. acidophilus milk daily. The physical character of the feces was also improved in the usual way. There was a striking reduction in the number of convulsions in 6 epilepties and a slight reduction in 3 others. These points will be discussed in detail in another communication.[5]

The microscopic examination of the gram-stained fecal smears showed an occasional preponderance of aciduric over proteolytic types. More significant are the results of actual numbers of L. acidophilus colonies appearing on whey agar plates. The percentage of L. acidophilus based on the total number of colonies appearing on the whey plates is shown in Table I. It will be remembered that in this investigation the calculations have been made on the dry basis, the 10 gm. fecal specimens having been air-dried.

TABLE I

ACTUAL NUMBER OF L. ACIDOPHILUS RECOVERED DURING TREATMENT (PER CENT)

| CASE NO. | 7/15 | 7/22 | 7/29 | 8/5 | 8/12 | 8/19 | 8/26 | 9/2 | 9/9 | 9/16 | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 21 | 66 | 50 | 82 | 37 | 12 | | | | 25 | 42 |
| 2 | | | 12 | | | 63 | | | 8 | | 28 |
| 3 | | 22 | 19 | 5 | | | | | 16 | 83 | 29 |
| 4 | 57 | | | 6 | | 40 | | 6 | 30 | | 28 |
| 5 | | | | | 65 | | 10 | 32 | 56 | | 41 |
| 6 | | | 50 | 30 | | | | 40 | 100 | | 55 |
| 7 | 42 | 9 | | | | | 30 | | 10 | | 23 |
| 8 | 100 | 12 | 7 | 55 | | 33 | 23 | 32 | 20 | 8 | 32 |
| 9 | 73 | | 53 | 34 | 10 | | | 86 | 4 | 5 | 38 |
| 10 | 57 | 80 | 60 | 60 | | | | | | | 64 |
| 11 | | | 24 | 24 | 50 | | 34 | | 40 | 50 | 37 |
| 12 | 84 | 49 | 48 | 48 | 22 | | 31 | 39 | 15 | 47 | 42 |
| 13 | | | 40 | 91 | 13 | | | 85 | · | | 57 |
| 14 | 24 | | | | 50 | | | | | | 37 |
| 15 | | | 13 | | | | | 50 | 92 | 12 | 42 |
| 16 | 92 | | | | 45 | | 15 | 8 | 16 | 40 | 36 |

From the data in Table I it will be seen that L. acidophilus appeared on the whey agar plates in varying amount. Only positive findings have here been recorded. At first glance it may seem that a comparatively low recovery of L. acidophilus was obtained. Since the patients received lactose for only two weeks, however, this is not surprising. Viable L. acidophilus have been recovered during treatment where none were previously found on plates of the highest dilutions. The causal relationship between viable L. acidophilus in the intestinal tract and an increase in normal defecations is thus again demonstrated.[1]

What influence have these viable L. acidophilus organisms on the colon-aerogenes group in the human intestine? This is the question of immediate concern. The difficulties in answering it satisfactorily are inherent in the problem. Unquestionably, the most important single factor is the composition and amount of food and fluid intake. In order to establish with any

degree of accuracy the normal number of colon-aerogenes organisms present in any individual, an extremely simple and rigorously controlled diet is essential. Even then considerable variation in numbers might be expected. Such variation was striking in the epileptics under consideration who were receiving the routine hospital diet. It was impossible to control their diet either in amount or composition because of the exigencies of ward management. The results in enumerating the colon-aerogenes organisms in these patients were, therefore, extremely variable.

It would scarcely be of value to publish the tables containing the data obtained. A typical instance alone will suffice. This is recorded in Table II and may be considered representative of the entire series of epileptics studied.

TABLE II

ACTUAL NUMBERS OF COLONIES ON WHEY AGAR AND COLON-AEROGENES MEDIUM FROM FECAL SPECIMENS OF A REPRESENTATIVE EPILEPTIC

(In Millions per Gram Dry Weight)

| TREATMENT | DATE | WHEY | L. ACIDOPHILUS | COLI |
|---|---|---|---|---|
| Nothing | 4/21 | 245.16 | | 60.00 |
| " | 4/29 | 664.89 | | 265.95 |
| " | 5/27 | 240.70 | | 111.60 |
| 1000 c.c. Sterile Milk Daily | 6/3 | 2141.38 | | 1070.68 |
| " " " " " | 6/24 | 1436.80 | | 104.10 |
| " " " " " | 7/1 | 3723.90 | | 797.87 |
| " " " " " | 7/8 | 625.00 | | 10.71 |
| 1000 c.c. L. Acidophilus Milk and 300 gm. Lactose Daily | 7/15 | 250.00 | 53.57 | 8.93 |
| 1000 c.c. L. Acidophilus Milk and 300 gm. Lactose Daily | 7/22 | 1428.50 | 952.30 | 104.76 |
| 1000 c.c. L. Acidophilus Daily | 7/29 | 2074.70 | 1037.35 | 95.43 |
| " " " " " | 8/5 | 1623.90 | 1346.10 | 85.47 |
| " " " " " | 8/12 | 1351.35 | 506.75 | 202.70 |
| " " " " " | 8/19 | 376.47 | 47.06 | 2.35 |
| " " " " " | 8/26 | 1320.75 | | 105.66 |
| " " " " " | 9/2 | 961.53 | | 60.43 |
| " " " " " | 9/9 | 491.40 | | 3.68 |
| " " " " " | 9/16 | 1434.85 | 359.71 | 59.31 |
| Nothing | 9/23 | 440.93 | | 22.22 |
| " | 9/30 | 1202.68 | | 23.38 |
| " | 10/14 | 174.53 | | 113.21 |
| " | 10/21 | 186.05 | | 158.91 |
| " | 10/28 | 192.11 | | 36.94 |
| " | 11/4 | 138.32 | | 78.68 |
| " | 11/18 | 514.52 | | 107.90 |

Averages of the actual numbers of the colon-aerogenes group found in individuals before, during, and after treatment have been compiled and are presented in Table III.

Generally speaking, it will be seen from Cases 1 through 12 in Table III that there is an increased number of colon-aerogenes group present when 1000 c.c. of sterile milk are ingested daily as compared with the period before administration. On the other hand, the administration of 1000 c.c. of L. acidophilus milk is responsible for a diminution in the numbers of the colon-aerogenes group when compared with the period during which the same amount of sterile milk was administered. When the period before treatment is compared with the period during which L. acidophilus was administered,

it will be seen that there are 2 cases showing a decrease and 7 cases showing an increase in the colon-aerogenes group. Comparing the period after treatment with that of L. acidophilus administration, it will be noted that there were almost as many cases showing an increase as there were cases showing a diminution in the colon-aerogenes group. The same is true in a comparison of numbers before and after treatment.

Considering the results of the second group of patients, Cases 13 through 24 who received 1000 c.c. L. acidophilus milk and 300 gm. lactose daily for eleven days, it will be seen from Table III that there were more instances of an increase than a decrease in the numbers of the colon-aerogenes group.

TABLE III

AVERAGE NUMBER OF COLON-AEROGENES GROUP BEFORE, DURING, AND AFTER TREATMENT
(Expressed in Millions per Gram on Dry Basis)

| CASE NO. | BEFORE TREATMENT | DURING 1000 C.C. STERILE MILK DAILY | DURING 1000 C.C. L. ACIDOPHILUS MILK DAILY | AFTER TREATMENT |
|---|---|---|---|---|
| 1 | 148 | 496 | 73 | 78 |
| 2 | 4 | 610 | 18 | 246 |
| 3 | 55 | 4191 | 166 | 26 |
| 4 | 22 | 407 | 61 | 216 |
| 5 | 69 | 601 | 36 | 39 |
| 6 | 2 | 34 | 82 | 65 |
| 7 | 18 | 1201 | 81 | 4 |
| 8 | 15 | 24 | 344 | 21 |
| 9 | 193 | 466 | 17 | 6 |
| 10 | 75 | 803 | 0 | – |
| 11 | 38 | 332 | 78 | 113 |
| 12 | 80 | 58 | 57 | 85 |
| 13 | 38 | | 19 | 88 |
| 14 | 1827 | | 1699 | 1780 |
| 15 | 426 | | 435 | 87 |
| 16 | 42 | | 11 | 102 |
| 17 | 249 | | 1 | 255 |
| 18 | 9 | | 1 | 235 |
| 19 | 17 | | 72 | 44 |
| 20 | 69 | | 2956 | 4736 |
| 21 | 5 | | 7 | 102 |
| 22 | 7 | | 2174 | 935 |
| 23 | – | | 6 | 121 |
| 24 | 17 | | 38 | 319 |

There were, however, more instances of an increase in these organisms after treatment was stopped than there was diminution. With one exception there were greater numbers of the colon-aerogenes group after than there were before treatment.

Averages compiled for the 12 patients receiving kaolin showed that in 9 cases there was an increase in the numbers of the colon-aerogenes group and that in 4 cases there was a diminution. Little importance need, therefore, be attached to kaolin as influencing the bacterial flora in these patients.

From the data presented it would be difficult to arrive at any valid conclusion concerning the influence of L. acidophilus on the colon-aerogenes group in the human intestine. Perhaps the most striking feature is that the ingestion of 1000 c.c. of sterile milk causes an increase in the numbers of these organisms. But a comparison of the period when L. acidophilus was administered with the period before and after treatment leaves much to be desired.

There is evidence that L. acidophilus does diminish the actual number of the colon-aerogenes group, but there is also evidence to the contrary. Undoubtedly, the large experimental error is chiefly responsible for the unsatisfactory nature of these results. The present report, therefore, which briefly outlines a mass of data, is perhaps suggestive rather than conclusive.

## SUMMARY

1. Weekly quantitative determinations of bacterial groups in fecal specimens were calculated upon the dry basis in a series of 37 epileptics over a period of five months. Selective media for the enumeration of the colon-aerogenes group and L. acidophilus were employed.

2. Twelve patients were fed 1000 c.c. L. acidophilus milk daily for two months after receiving 1000 c.c. of sterile milk for a month. Twelve patients received 1000 c.c. of L. acidophilus milk and 300 gm. of lactose daily. Thirteen patients received 60 gm. kaolin daily for two months and an additional amount up to 240 gm. daily for one month. Daily records were kept of convulsions and defecations. For one month before, and for two months after treatment, as well as during treatment, observations and fecal analyses were carried out.

3. L. acidophilus was recovered in varying amount from all patients ingesting L. acidophilus milk for two months. No L. acidophilus was recovered from patients receiving kaolin.

4. Averages of the numbers of the colon-aerogenes group indicated an increase when sterile milk was administered. Compared with this, L. acidophilus caused a diminution. When, however, the period during which L. acidophilus was administered was compared with the periods before and after treatment, diminution in numbers of the colon-aerogenes group occurred in some instances and an increase in others. Kaolin gave similar but more unfavorable results.

5. Then experimental error in this investigation was so great as to preclude clear-cut conclusions.

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# STUDIES ON THE AUTONOMIC SYSTEM, III. THE REACTION OF THE CIRCULAR MUSCLE OF THE EXCISED SURVIVING SMALL INTESTINE OF THE FROG (RANA PIPIENS) TO EPINEPHRIN, PILOCARPIN, ATROPINE, AND BARIUM.*

By George B. Roth, A.B., M.D., Washington, D.C.

## INTRODUCTION

THE reaction of the excised surviving longitudinal muscle of the small intestine of the frog (Rana pipiens) to epinephrin, pilocarpin, atropine, and barium was previously reported[1] to be at variance with the results obtained in mammals as reported by previous investigators.

In the following report the effect of the above-mentioned poisons on the circular muscle of the excised surviving small intestine of the frog (Rana pipiens) will be considered.

## METHOD OF STUDY

In order to investigate the effect of the above poisons on the circular muscle of the small intestine of the frog, rings measuring from two to three millimeters in length were taken from pithed frogs, about a half inch above the rectum and from a portion of the intestine situated at a similar distance below the stomach. For the sake of comparison, in many instances a longitudinal segment measuring about 10 to 15 mm. was taken from the region immediately below the upper circular ring or above the lower circular ring and mounted with the rings. Both circular rings and the longitudinal segment were then arranged in the usual manner in oxygenated Locke-Ringers solution so as to obtain permanent graphic records. The rings were suspended intact, similar to rubber bands and not as strips. Further details as to the method may be found in the first paper of this series. (See Footnote 1.)

Table I

Saline Solutions

| Locke-Ringers Solution | NaCl | KCl | $CaCl_2$ anhydrous | $NaHCO_3$ | $NaH_2PO_4$ |
|---|---|---|---|---|---|
| | per cent | per cent | per cent | per cent | per cent |
| I | 0.7 | 0.02 | 0.02 | – | – |
| II | 0.8 | 0.02 | 0.02 | – | – |
| III | 0.7 | 0.02 | 0.02 | 0.05 | – |
| IV | 0.7 | 0.02 | 0.02 | 0.1 | 0.005 |

*From the Pharmacological Laboratory of Western Reserve University School of Medicine, Cleveland, Ohio, and the Laboratory of Physiology and Pharmacology, George Washington University School of Medicine, Washington, D. C.

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[1]Roth, George B. "Studies on the autonomic system, I. The antagonism of the stimulant action of barium chloride on the excised surviving small intestine of the frog (Rana pipiens) by means of epinephrin, pilocarpin and atropine. Arch. internat. de pharmacod. et de therap., 1923, xxvii, 333.

Experiments were made with several saline solutions; their composition will be found in Table I. It was found that the alkaline solutions were best suited for the development of rhythm; therefore they were used in the majority of the experiments.

Fig. 1.—(R83D1) Effect of a small dose of epinephrin on the circular muscle of the small intestine of R. pipiens. Oxygenated Locke-Ringers solution No. 1 at 23° C. was employed as the saline solution.

## RESULTS OBTAINED

The results obtained in this investigation were not as clear cut as those obtained previously on the longitudinal muscle; perhaps in the present study this was caused by using a saline which permitted rhythm. It was exceedingly

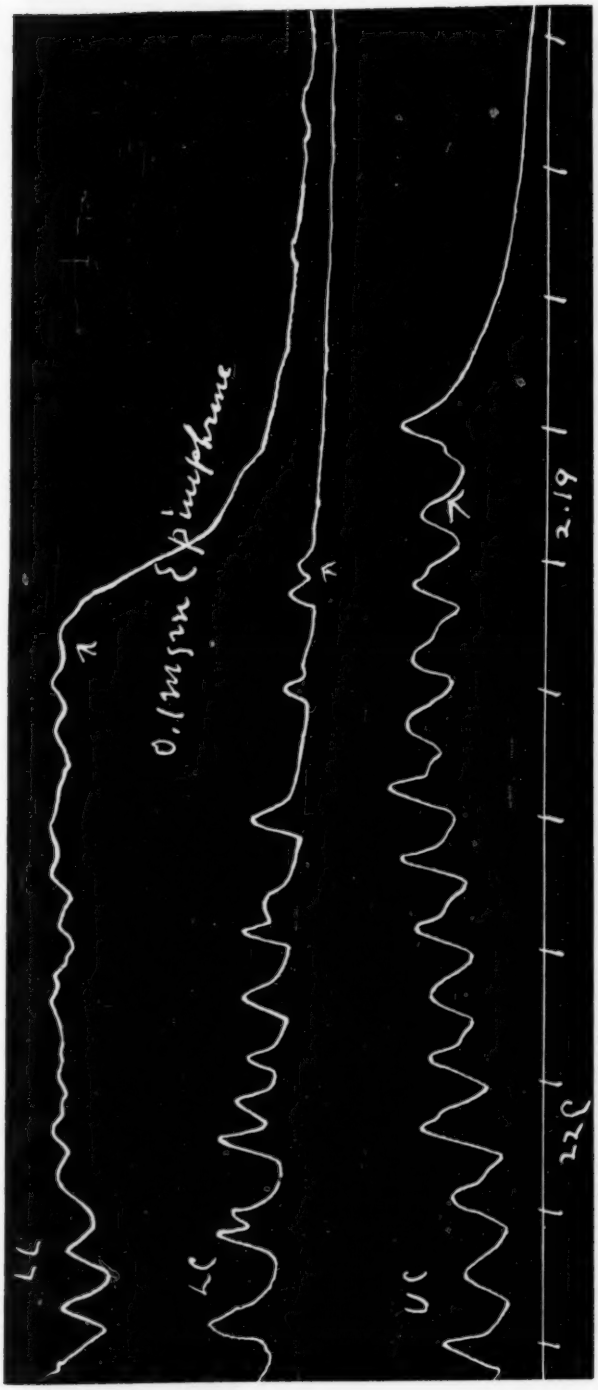



Fig. 2.—(R118D3) Effect obtained from the use of a relatively high concentration of epinephrin on the longitudinal (*LL*) and circular (*LC* and *UC*) muscles of the excised small intestine of R. pipiens. Oxygenated Locke-Ringers No. 3 at 22° C. was used as the saline solution.

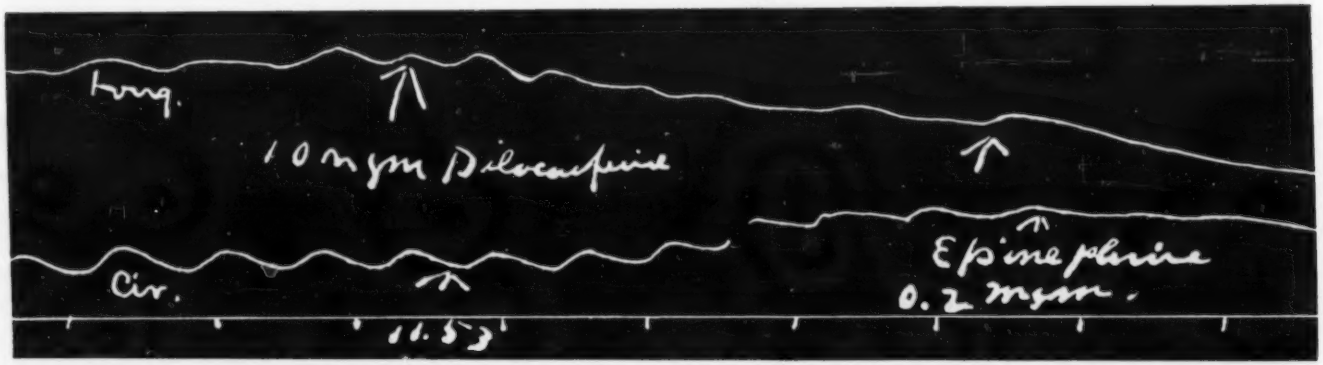



Fig. 3.—(R76D1) Response of longitudinal (*Long.*) and circular (*Cir.*) muscles of the excised small intestine of R. pipiens to 10 mg. of pilocarpin, oxygenated Locke-Ringers solution No. 1at 22° C. being used as the saline solution.

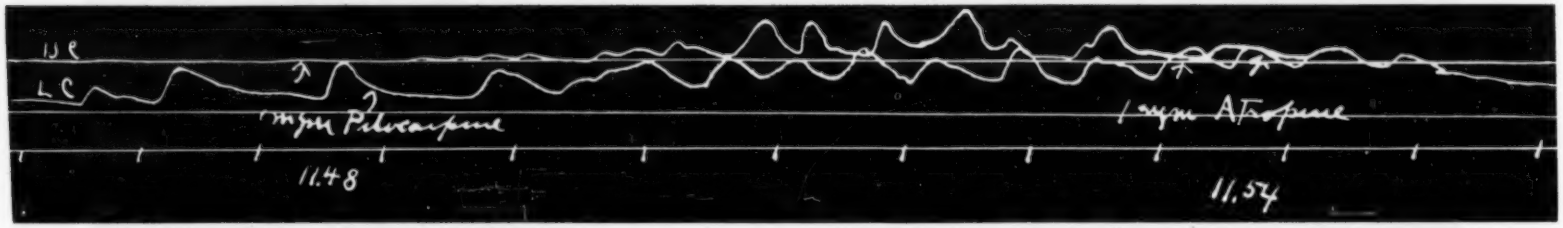



Fig. 4.—(R98D2) Antagonistic action of 1 mg. of atropine against 1 mg. of pilocarpin on the circular muscle of the excised small intestine of R. pipiens when suspended in oxygenated Locke-Ringers solution No. 4 at 19° C.

difficult, therefore, in some cases to rule out spontaneous changes in rhythm, in tonus, or in the amplitude of contraction.

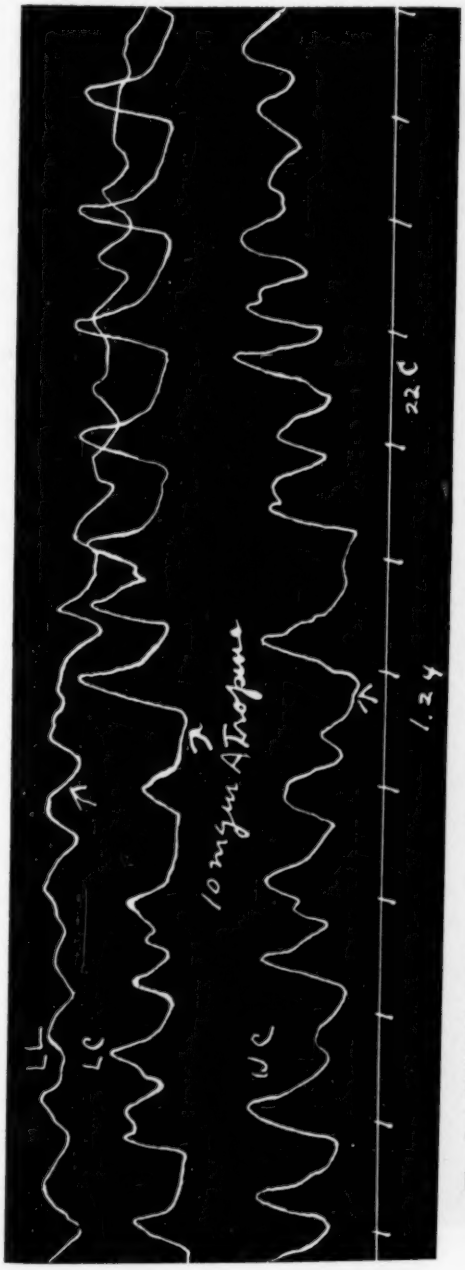



Fig. 5.—(R118D2) Response of longitudinal (*LL*) and circular muscles (*LC* and *UC*) of the excised small intestine of R. pipiens to 10 mg. of atropine when suspended in oxygenated Locke-Ringers solution No. 3 at 22° C. Tonus decreases in the longitudinal (*LL*) and increases in the circular (*LC* and *UC*) preparations.

EPINEPHRIN

The effects obtained from epinephrin depended somewhat upon dosage; that is, 0.01 mg. may increase tonus and the height of rhythmic contraction

or may cause rhythm to appear in a quiescent ring. In other instances 0.01 mg. may depress tonus and abolish rhythm.[2]

In concentrations greater than 0.01 mg. but not exceeding 1 mg., a decrease in tonus with abolition of rhythm usually occurred. Out of 37 experi-

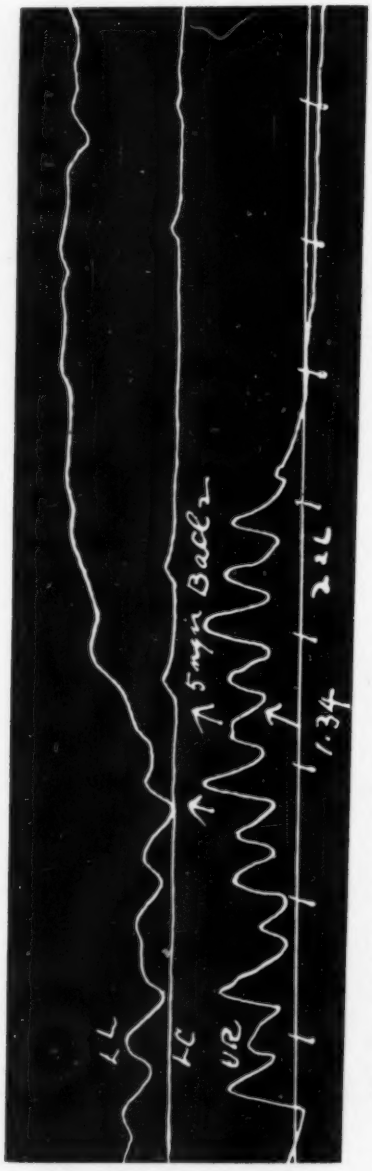



Fig. 6.—(R122D2) Effect of 5 mg. of barium chloride on the longitudinal (*LL*) and circular (*LC* and *UC*) muscles of the excised small intestine of R. pipiens when suspended in Locke-Ringers solution No. 3 at 22°C. Tonus increases in longitudinal, rhythm appears in lower circular, while tonus decreases and rhythm ceases in upper circular preparation.

ments in which epinephrin was used, negative results were obtained in 9 instances. (See Figs. 1 and 2.)

[2]The quantity of drug referred to in these experiments indicates the amount added to 100 c.c. of the saline solution; for example, when 0.01 mg. was employed, the concentration bathing the tissues was therefore a 1 to 100,000 solution.

### PILOCARPIN

In dosages ranging from 0.1 mg. to 10 mg., pilocarpin was found either to produce an increase in tonus, in amplitude, and occasionally in rhythm in the circular muscle of the small intestine of the frog, or to have no effect. (See Figs. 3 and 4.)

As a rule the longitudinal segments which were employed in this investigation for comparison reacted in the same manner to that previously reported, namely, to produce depression. (See Fig. 3.) In 4 out of 33 experiments, however, in which 1 mg. was used, a slight increase in tonus occurred in the longitudinal segment. An increase in tonus likewise occurred in one experiment, after 0.1 and 10 mg. dosages. Apparently these results are due to the fact that in the present investigation the saline employed permitted response to weak stimulating influences.

### ATROPINE

Atropine in dosages ranging from 0.1 to 20 mg. produced variable results on the circular muscle of the small intestine of the frog. Usually no response was obtained, but in a few experiments an increase in tonus, amplitude and rhythm occurred (see Fig. 5), while in other experiments a slight decrease in tonus, amplitude, and rhythm was produced. Atropine in equal dosage was able to completely antagonize the stimulating action of pilocarpin. (See Fig. 4.)

### BARIUM

The experiments with barium chloride on the circular muscle of the excised small intestine of the frog yielded results similar to those with atropine, namely, no response or in a few experiments either stimulation or depression was obtained. The dosages employed ranged from 0.1 mg. to 20 mg. The results were independent of dosage. In Fig. 6, for example, 5 mg. caused rhythm to appear in the lower circular ring, while in the upper one tonus was markedly depressed and rhythm disappeared.

### SUMMARY

The response of the circular muscle of the excised surviving small intestine of the frog (Rana pipiens) to epinephrin, pilocarpin, atropine, and barium is essentially the same as that of the longitudinal muscle; the principal difference is that pilocarpin is primarily a stimulant to the circular ring, whereas it is mainly a depressant to the longitudinal muscle. This phenomenon is believed to be unique. Epinephrin in small doses may act as a stimulant or depressant to tonus, rhythm, and amplitude of contraction, while more concentrated solutions are usually depressant. Pilocarpin is primarily a stimulant. Atropine is usually ineffective but may be either stimulant or depressant. It is able to antagonize pilocarpin completely in equal dosage. Barium, like atropine, usually yielded negative results but in a few experiments either stimulation or depression was obtained. The results were independent of dosage.

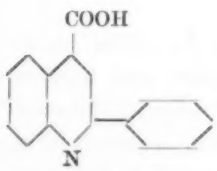
# THE EFFECT OF URIC ACID ELIMINANTS ON THE URIC ACID EXCRETION OF DALMATIAN DOGS*

By Edward E. Swanson, Indianapolis, Ind.

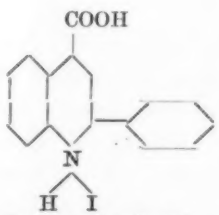
## I. REVIEW OF LITERATURE

BENEDICT[1] in 1916 was the first to report that the Dalmatian breed of dog commonly known as the carriage or coach dog excretes a greater amount of uric acid than any other breed of dog. Wells[2] confirmed his findings. Onslow,[3] being interested in the percentage of uric acid of the offspring from a cross between the Dalmatian and a normal dog, found that in general the hybrid Dalmatian excreted relatively the same amount of uric acid and allantoin as a normal dog. In some cases the spotted hybrid animal may excrete a slight excess over the nonspotted hybrid dog. The physiologic power or capacity for destroying uric acid has been expressed as the *uricolytic index,* or the quantitative ratio of allantoin nitrogen to the sum of allantoin nitrogen and uric acid nitrogen. Hunter and Givens,[4] Hunter, Givens, and Guion,[5] Hunter and Ward,[6] and later Onslow[3] found the uricolytic index of the majority of mammals to be about 98, but in some it has been found to be as low as 79 to 80. The uricolytic index of man is about 2. Benedict[1] found that the uricolytic index of the Dalmatian dog varied from 24 to 62 according to the diet. Folin, Berglund, and Derick[7] found that in hybrid dogs the destruction of uric acid seems to take place in the circulating blood, while in the case of the coach dog there appears to be less destruction in the circulating blood and a much greater excretion through the kidney.

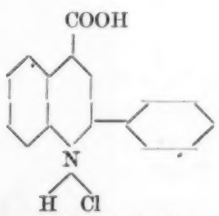
The effect of quinolin carboxylic on the uric acid metabolism was first investigated by Nicolaier.[8] Some derivatives of this compound studied are:



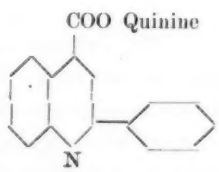

2—phenyl-quinolin 4—carboxylic acid (Phenylcinchoninic acid)




2—phenyl-quinolin 4—carboxylic acid hydriodid (Oxyliodid)




2—phenyl-quinolin 4—carboxylic acid hydrochlorid (Chloroxyl)




Quinine Phenylcinchoninate (Quinoxyl)

According to Cushny, the administration of these compounds does not change the amount of urine excreted. It is often turbid when passed and deposits quantities of urates on standing, no other constituent of the urine

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being altered appreciably in amount. This increased elimination of urates and uric acid occurs on ordinary diets and also on a purine-free diet, in which case the uric acid excreted can arise only from the tissue metabolism. Folin and Lyman[9] found that the more rapid excretion of uric acid is attended by a fall in the uric acid content of the blood, which indicates a direct action on the kidney and is more readily permeated by the urates.

The value of compounds of the quinolin carboxylic acid series in uric acid metabolism being generally known in clinical practice, it occurred to us that the Dalmatian dog might be used as a test animal to determine the value of these drugs as uric acid eliminators.

## II. METHOD OF TECHNIC

*A. The uric acid metabolism of the Dalmatian dog.*—Four Dalmatian dogs were tested for several days in a standard type of metabolism cage, urine collected each morning and the uric acid percentage determined. The diet was well-cut fresh meat mixed with bone-ash.

The results of each test are shown as follows:

Coach Dog 1
Female—Wt. = 14.6 Kg.

| DATE | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET PER DAY |
|---|---|---|---|
| 9–3 | 305 c.c. | 0.227 | 250 gm. + 3 gm. Bone-ash. |
| 9–4 | 325 c.c. | 0.323 | 250 gm. + 3 gm. Bone-ash. |
| 9–5 | 280 c.c. | 0.260 | 250 gm. + 3 gm. Bone-ash. |
| 9–6 | 670 c.c. | 0.300 | 250 gm. + 3 gm. Bone-ash. |

Coach Dog 2
Female—Wt. = 10.4 Kg.

| DATE | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET PER DAY |
|---|---|---|---|
| 9–3 | 860 c.c. | 0.391 | 250 gm. + 3 gm. Bone-ash. |
| 9–4 | 665 c.c. | 0.405 | 250 gm. + 3 gm. Bone-ash. |
| 9–5 | 860 c.c. | 0.411 | 250 gm. + 3 gm. Bone-ash. |
| 9–6 | 645 c.c. | 0.566 | 250 gm. + 3 gm. Bone-ash. |

Coach Dog 3
Male—Wt. = 21 Kg.

| DATE | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET PER DAY |
|---|---|---|---|
| 8–28 | 420 c.c. | 0.119 | 250 gm. + 3 gm. Bone-ash. |
| 8–29 | 285 c.c. | 0.329 | 250 gm. + 3 gm. Bone-ash. |
| 8–30 | 280 c.c. | 0.348 | 250 gm. + 3 gm. Bone-ash. |
| 8–31 | 285 c.c. | 0.129 | 250 gm. + 3 gm. Bone-ash. |

Coach Dog 4
Male—Wt. = 14 Kg.

| DATE | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET PER DAY |
|---|---|---|---|
| 9–9 | 400 c.c. | ---- | 250 gm. + 3 gm. Bone-ash. |
| 9–10 | 300 c.c. | ---- | 250 gm. + 3 gm. Bone-ash. |
| 9–11 | 415 c.c. | 0.010 | 250 gm. + 3 gm. Bone-ash. |
| 9–12 | 310 c.c. | 0.010 | 250 gm. + 3 gm. Bone-ash. |

Three of the 4 dogs gave distinct uric acid excretion. Benedict[1] observed 4 out of 5 dogs to be positive; Watanabe[10] exhibited 2 but did not get as clear results, an evidence perhaps of not using a pure breed. Wells[2] and Givens[4, 5] found positive results in all of their dogs.

*B. Preparation of a Standard Diet.*—The uric acid metabolism of the dogs having been determined, the 3 positive dogs were prepared for more careful treatment.

A standard diet was prepared as follows for a 15 kg. dog:

| | |
|---|---|
| Fresh meats | 250 gm. |
| Dog Biscuits | 60 gm. |
| Lard | 30 gm. |
| Bone-ash | 10 gm. |
| | 350 gm. |

This formula was prepared in large quantities as follows: the biscuits and meat were ground with a meat grinder, the lard and bone-ash added, mixed thoroughly, and finally the whole mass was run a second time through the grinder. For each quantity prepared, the nitrogen content was determined.

The dogs were weighed, placed in metabolism cages, fed once a day (in the morning), urine collected every twenty-four hours (in the morning), and the percentage of uric acid determined by the Hopkins Method, Folin's modification, Simon, *Clinical Diagnosis*, p. 378. The drugs were given when the dogs maintained a constant uric acid excretion.

*C. Protocols.*—Following are the results of the experiments:

Dog 1

Female—Wt. = 12.6 Kg.

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 6– 4 | | 675 c.c. | 0.360 | 300 gm. | 3.30 |
| 6– 5 | | 520 c.c. | 0.388 | " " | " |
| 6– 6 | | 275 c.c. | 0.380 | " " | " |
| 6– 7 | | 475 c.c. | 0.310 | " " | " |
| 6– 8 | | 465 c.c. | 0.312 | " " | " |
| 6– 9 | | 425 c.c. | 0.336 | " " | " |
| 6–10 | Phenylcinchoninic | 280 c.c. | 0.337 | " " | " |
| 6–11 | Hydrochlorid | 515 c.c. | 0.620 | " " | " |
| 6–12 | (Chloroxyl) 1 gm. | 320 c.c. | 0.420 | " " | " |
| 6–13 | | 290 c.c. | 0.360 | " " | " |

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 6–17 | | 390 c.c. | 0.357 | 300 gm. | 2.83 |
| 6–18 | | 400 c.c. | 0.355 | " " | " |
| 6–19 | Phenylcinchoninic | 185 c.c. | 0.365 | " " | " |
| 6–20 | Hydriodid | 610 c.c. | 0.523 | " " | " |
| 6–21 | (Oxyliodid) 1 gm. | 270 c.c. | 0.280 | " " | " |
| 6–22 | | 450 c.c. | 0.323 | " " | " |
| 6–23 | | 365 c.c. | 0.368 | " " | " |
| 6–24 | Phenylcinchoninic | 325 c.c. | 0.300 | " " | " |
| 6–25 | acid 1 gm. | 420 c.c. | 0.412 | " " | " |
| 6–26 | | 235 c.c. | 0.356 | " " | " |
| 6–27 | | 225 c.c. | 0.193 | " " | " |

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 10–21 | | 250 c.c. | 0.524 | 300 gm. | 3.72 |
| 10–22 | | 500 c.c. | 0.526 | " " | " |
| 10–23 | | 190 c.c. | 0.469 | " " | " |
| 10–24 | | 330 c.c. | 0.471 | " " | " |
| 10–25 | | 140 c.c. | 0.549 | " " | " |
| 10–26 | | 485 c.c. | 0.551 | " " | " |
| 10–27 | | 200 c.c. | 0.516 | " " | " |
| 10–28 | | 375 c.c. | 0.514 | " " | " |

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 10–29 | | 225 c.c. | 0.400 | 300 gm. | 3.72 |
| 10–30 | Phenylcinchoninic | 160 c.c. | 0.430 | " " | " |
| 10–31 | Hydrochlorid | 450 c.c. | 0.853 | " " | " |
| 11– 1 | (Chloroxyl) 1 gm. | 250 c.c. | 0.620 | " " | " |
| 11– 2 | | 250 c.c. | 0.450 | " " | " |
| 11– 3 | | 220 c.c. | 0.480 | " " | " |
| 11– 4 | | 250 c.c. | 0.480 | " " | " |
| 11– 5 | Phenylcinchoninic | 260 c.c. | 0.400 | " " | " |
| 11– 6 | Hydriodid | 360 c.c. | 0.610 | " " | " |
| 11– 7 | (Oxyliodid) 1 gm. | 230 c.c. | 0.450 | " " | " |
| 11– 8 | | 300 c.c. | 0.480 | " " | " |

Dog 2
Female—Wt. = 15 Kg.

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 6–14 | | 530 c.c. | 0.292 | 350 gm. | 2.83 |
| 6–15 | | 515 c.c. | 0.288 | " " | " |
| 6–16 | | 575 c.c. | 0.300 | " " | " |
| 6–17 | | 525 c.c. | 0.280 | " " | " |
| 6–18 | | 515 c.c. | 0.235 | " " | " |
| 6–19 | Phenylcinchoninic | 475 c.c. | 0.233 | " " | " |
| 6–20 | Hydriodid | 510 c.c. | 0.460 | " " | " |
| 6–21 | (Oxyliodid) 1 gm. | 650 c.c. | 0.471 | " " | " |
| 6–22 | | 500 c.c. | 0.367 | " " | " |
| 6–23 | | 470 c.c. | 0.283 | " " | " |

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 12 –6 | | 525 c.c. | 0.360 | 350 gm. | 3.67 |
| 12– 7 | | 415 c.c. | 0.370 | " " | " |
| 12– 8 | | 790 c.c. | 0.350 | " " | " |
| 12– 9 | | 275 c.c. | 0.390 | " " | " |
| 12–10 | | 335 c.c. | 0.340 | " " | " |
| 12–11 | Phenylcinchoninic | 390 c.c. | 0.390 | " " | " |
| 12–12 | Hydrochlorid | 610 c.c. | 0.620 | " " | " |
| 12–13 | (Chloroxyl) 1 gm. | 360 c.c. | 0.320 | " " | " |
| 12–14 | | 440 c.c. | 0.430 | " " | " |
| 12–15 | | 370 c.c. | 0.390 | " " | " |
| 12–16 | Phenylcinchoninic | 475 c.c. | 0.390 | " " | " |
| 12–17 | Hydriodid | 310 c.c. | 0.430 | " " | " |
| 12–18 | (Oxyliodid) 1 gm. | 380 c.c. | 0.380 | " " | " |
| 12–19 | | 500 c.c. | 0.430 | " " | " |
| 12–20 | Quinine Phenyl- | 415 c.c. | 0.430 | " " | " |
| 12–21 | cinchoninate | 650 c.c. | 0.570 | " " | " |
| 12–22 | (Quinoxyl) 1 gm. | 360 c.c. | 0.370 | " " | " |
| 12–23 | | 525 c.c. | 0.370 | " " | " |
| 12–24 | | 435 c.c. | 0.370 | " " | " |
| 12–25 | | 315 c.c. | 0.370 | " " | " |
| 12–26 | Phenylcinchoninic | 400 c.c. | 0.370 | " " | " |
| 12–27 | acid 1 gm. | 650 c.c. | 0.560 | " " | " |
| 12–28 | | 450 c.c. | 0.550 | " " | " |
| 12–29 | | 400 c.c. | 0.450 | " " | " |
| 12–30 | | 425 c.c. | 0.380 | " " | " |

Dog 3
Male—Wt. = 21 Kg.

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 10–25 | | 315 c.c. | 0.510 | 400 gm. | 3.72 |
| 10–26 | | 485 c.c. | 0.570 | " " | " |
| 10–27 | | 410 c.c. | 0.490 | " " | " |
| 10–28 | | 360 c.c. | 0.530 | " " | " |
| 10–29 | | 340 c.c. | 0.550 | " " | " |
| 10–30 | | 325 c.c. | 0.520 | " " | " |
| 10–31 | | 265 c.c. | 0.420 | " " | " |

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 11- 1 | | 325 c.c. | 0.340 | 400 gm. | 3.72 |
| 11- 2 | Phenylcinchoninic | 225 c.c. | 0.270 | " " | " |
| 11- 3 | Hydrochlorid | 350 c.c. | 0.560 | " " | " |
| 11- 4 | (Chloroxyl) 1 gm. | 270 c.c. | 0.340 | " " | " |
| 11- 5 | | 225 c.c. | 0.350 | " " | " |
| 11- 6 | | 230 c.c. | 0.490 | " " | " |

| DATE | DRUG | AMOUNT OF URINE | URIC ACID PERCENTAGE | DIET | NITROGEN PERCENTAGE |
|---|---|---|---|---|---|
| 11-15 | | 275 c.c. | 0.370 | 400 gm. | 3.29 |
| 11-16 | | 360 c.c. | 0.330 | " " | " |
| 11-17 | | 315 c.c. | 0.360 | " " | " |
| 11-18 | Phenylcinchoninic | 300 c.c. | 0.320 | " " | " |
| 11-19 | acid 1 gm. | 325 c.c. | 0.480 | " " | " |
| 11-20 | | 970 c.c. | 0.460 | " " | " |
| 11-21 | | 1300 c.c. | 0.160 | " " | " |
| 11-22 | | 1075 c.c. | 0.200 | " " | " |
| 11-23 | | 975 c.c. | 0.160 | " " | " |
| 11-24 | | 750 c.c. | 0.190 | " " | " |

Dog 1 gave a constant normal uric acid value. Following the oral administration of 1 gm. of phenylcinchoninic hydrochlorid (chloroxyl), the uric acid content increased from a normal average of 0.346 to 0.620 gm. in twenty-four hours and returned to normal in forty-eight hours. One gram of phenylcinchoninic hydriodid (oxyliodid) and 1 gm. of phenylcinchoninic acid, respectively, were given and produced a distinct increase in the uric acid excretion. One gram of phenylcinchoninic hydrochlorid (chloroxyl) and 1 gm. of phenylcinchoninic hydriodid (oxyliodid), respectively, again repeated the same distinct increase in uric acid output.

Dog 2, following a constant normal uric acid value, was given the same drugs, also quinine phenylcinchoninate (quinoxyl). These drugs all produced a similar increase in uric acid excretion.

Dog 3 was given 1 gm. of phenylcinchoninic hydrochlorid (chloroxyl) and phenylcinchoninic acid. Each drug produced a distinct increase in the uric acid content.

## III. CONCLUSION

1. The excretion of uric acid of the Dalmatian Coach dog is comparable to that of the human uric acid metabolism.

2. The uric acid eliminants produce a distinct increase in the uric acid excretion.

3. It is possible to determine the value of these drugs as uric acid eliminators by the percentage of uric acid excretion of Dalmatian dogs.

The writer is much indebted to Dr. A. L. Walters and Miss Lucile Carter for their suggestions and criticisms.

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# A COMPARISON OF THE CLEVELAND AND THE KOLMER MODIFICATIONS OF THE WASSERMANN TEST*

By Frank R. Lyne, M.D., Cleveland, Ohio

IN 1924 the Cleveland Academy of Medicine appointed a committee, with Dr. H. N. Cole as chairman, to investigate the various modifications of the Wassermann test in common use. This was done with the idea of recommending as a standard system some one method to the laboratories of Cleveland. As a result of the activities of this committee a method was devised which has been called the Cleveland modification.[1, 2] A brief résumé of that method will be given here, emphasizing those points in which it differs from others now in vogue.

The method for preparing antigen was devised by Ecker.[3] Thirty grams of dried beef heart are extracted with 150 c.c. acetone in a reflux condenser over a water-bath for four hours. The mixture is chilled, filtered, and the powder dried. This is now extracted in the same apparatus with 150 c.c. acetone-free, methyl alcohol for the same period of time. The mixture is now filtered while hot and the filtrate evaporated to 90 c.c. For use, the antigen is diluted to one-fourth its anticomplementary unit by adding 0.9 per cent saline solution drop by drop to the proper amount of extract (usually about 1:30). I prefer to reinforce the antigen with 0.2 per cent cholesterol.[1]

Sheep cells are sensitized by adding to 50 c.c. of 5.0 per cent cell suspension, 50 c.c. of a dilution of antisheep hemolysin containing 1000 units; thus each 0.5 c.c. of the mixture contains 5 units. The cell suspension is rotated while adding the hemolysin, and the mixture is allowed to stand in the water-bath at 37° C. for thirty minutes before use. One-half c.c. of the mixture is used in each tube of the complement titration and of the reactions.

Complement is the pooled serum of guinea pigs. It is titrated by placing in a series of tubes the following amounts of 1:30 dilution: 0.09 c.c., 0.12 c.c., 0.15 c.c., etc., to 0.30 c.c. One-half c.c. of sensitized cell suspension is added, then the volume of each tube is brought to 1.5 c.c. with saline solution. The tubes are placed in the water-bath at 37° C. for thirty minutes. The titer is clear-cut and easily read, the unit being the least amount that produces complete hemolysis. Two units, in either 1:10 or 1:15 dilution, are used in the reactions.

Serums to be tested are inactivated at 56° C. for twenty-five minutes. Titration of other reagents used has been given elsewhere.[1]

Reactions are set up as follows: three tubes are arranged for each serum. Tube No. 1 contains 0.1 c.c. serum, tube No. 2 contains 0.05 c.c., and

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No. 3 which is the control contains 0.1 c.c. Tubes Nos. 1 and 2 now receive 0.1 c.c. of freshly prepared antigen dilution; the contents are mixed and allowed to remain at room temperature for fifteen minutes. Two units of complement dilution are added to each tube, and the tubes are placed in the ice box at 6° to 8° C. for fifteen to eighteen hours. Nine-tenths per cent saline solution is then added to each tube to a volume of 1.0 c.c., and 0.5 c.c. sensitized cell suspension is added when the tubes are placed in the water-bath at 37° C. for thirty minutes to permit hemolysis. Readings may be made at once, but preferably after partial sedimentation has taken place.

The Cleveland modification has several advantages. Under proper conditions of preservation the hemolysin does not require titration more often than every six weeks or two months. The complement titrations give clear-cut end points. The small total volume (1.5 c.c.) in both titrations and tests saves reagents and increases the velocity of the reactions. The use of large doses of hemolysin (5 units) renders unnecessary daily titration, increases the velocity of the reactions, especially in the complement titration, and obviates those false negatives due to the presence of native antisheep hemolysis in human serum.[1] In spite of the fact that this introduces a large amount of rabbit serum into the reaction, this is more than compensated by the reduction in the amount of guinea pig serum. Thus the total amount of foreign serum is materially reduced.

Probably the most widely employed modification of the Wassermann test is that of Kolmer. This method is sensitive, specific, and has given satisfactory results in many laboratories. It differs from the Cleveland method in a number of respects, notably in the daily titration of hemolysin, the use of only two units of hemolysin, a weaker cell suspension, and the use of unsensitized cells, and that it requires about twice as much complement for the same number of reactions. The Kolmer technic used in this department is the qualitative method outlined in "Laboratory Diagnostic Methods."[4]

Through the kindness of Dr. Kolmer the writer spent some time in his laboratory studying the method under his supervision, and the antigen used in the comparative tests described herein was provided by him.

The comparison of the Kolmer and the Cleveland modifications was carried out on a series of one thousand consecutive sera received in the laboratory. These were from the Medical Department of St. Alexis Hospital, Dr. Richard Dexter, director, and from the Department of Dermatology of the Cleveland City Hospital, Dr. H. N. Cole, director. Of these sera, 680 were negative in both; 245 gave equally positive reactions. The Kolmer system gave stronger results with 20, while the Cleveland system gave stronger results with 38.

Where one system was positive and the other negative, 3 were positive in the Kolmer system, 2 of these being known syphilitic cases under treatment; 11 were positive in the Cleveland system, 9 being known syphilitic cases. Seventeen sera were anticomplementary in the Kolmer, 15 in the Cleveland system. These results may be tabulated as follows:

Negative in both systems: 680
Equally positive in both: 245
Stronger positive results in the Kolmer system: 20
Stronger positive results in the Cleveland: 38
Positive in the Kolmer, negative in the Cleveland system: 3 (2 known syphilitic)
Positive in the Cleveland, negative in the Kolmer system: 11 (9 known syphilitic)
Anticomplementary in the Kolmer: 17
Anticomplementary in the Cleveland: 15.

It is obvious that there is no significant difference in sensitivity and specificity between the two systems.

In the above study an antigen reinforced with 0.2 per cent cholesterol was used in the Cleveland system. Using an antigen of the same type, but without reinforcement with cholesterol, the following results were obtained:

Negative in both systems: 680
Equally positive in both: 225
Stronger positive results in the Kolmer system: 50
Stronger positive results in the Cleveland: 28
Positive in the Kolmer, negative in the Cleveland system: 5 (4 known syphilitic)
Positive in the Cleveland, negative in the Kolmer: 7 (5 known syphilitic)
Anticomplementary in the Kolmer: 17
Anticomplementary in the Cleveland: 15.

Here again it is apparent that there is no significant variation between the two systems. It should be remembered, however, that the Kolmer antigen contains 0.2 per cent cholesterol.

The Cleveland modification, then, is as sensitive and as specific as the Kolmer. The points of advantage that recommend the former most highly have been mentioned. It offers a decided saving in time, for the time consumed in the titration of reagents each day the test is done is only the thirty minute incubation used in the complement titration. This titer is always clear-cut and the results unmistakable, which often is not the case with the hemolysin and complement titrations using the Kolmer technic with the same reagents. The amount of complement used in the tests is about half that required for the same number of sera in the Kolmer system; this is an important item, especially in smaller laboratories where guinea pigs are bled from the heart. Larger amounts of hemolysin are used, it is true; but this is not of such practical importance, since we are able to preserve this reagent in large amounts over a long period of time. Positive reactions are not obscured by excess native antisheep hemolysin in the patient's serum. A very decided advantage is that the preparation of antigen is readily carried out, requiring only two periods of extraction of four hours each—the product is easily completed in two days. Anticomplementary reactions are encountered in about the same proportion in both systems.

## SUMMARY

An analysis of results obtained with 1000 consecutive sera submitted to the Wassermann reaction using both the Kolmer and the Cleveland modifications shows them to be equal in sensitivity and specificity.

The Cleveland modification is uninfluenced by native antisheep hemolysin often present in human serum, is decidedly economical as to time and reagents, and for these reasons should be the method chosen.

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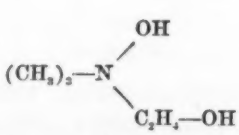
# UNION BETWEEN ANTIGEN AND ANTIBODY IN THE WASSERMANN TEST*

BY S. L. LEIBOFF, A.M., NEW YORK CITY

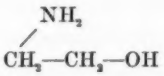
THE question whether the antigen used in the Wassermann test for syphilis unites chemically with the specific substance or substances present in syphilitic serum, or whether there is a mere change in the physical state of the colloids, has never been proved. We know almost nothing about the specific substances present in syphilitic serum. We do know, however, something about the nature of the antigen. Noguchi[1] showed it to be a phosphatid. Porges and Myer[2] believed the particular phosphatid to be lecithin. Sachs and Rondoni[3] used synthetic antigens which contained lecithin as a basis; however, the results obtained were not as good as those obtained with alcoholic liver extracts. Most likely alcoholic liver extracts contain a mixture of various phosphatids. According to MacLean[4] a great number of substances regarded as distinct phosphatids are only mixtures of various lipoids, since it is very difficult to isolate lipoidal substances in a chemically pure form. This difficulty is further complicated by the property of being intersoluble which these bodies possess.

There are four phosphatids as to whose existence as chemical individuals there is definite evidence; viz., lecithin, cephalin, cuorin, and sphingomyelin. They are characterized by containing phosphorus, nitrogen, and fatty acids; they are insoluble in acetone; they form compounds with cadmium chloride by which they are precipitated; they combine with proteins;[4] on hydrolysis they yield phosphoric acid (in the case of the first three phosphatids, in the form of glycerophosphoric acid), various fatty acids, and basic bodies such as cholin:

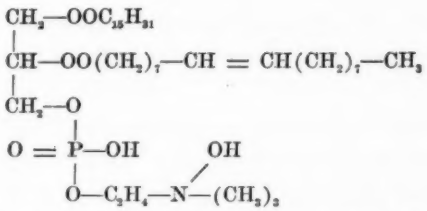



*From the Biochemical Department of Lebanon Hospital Laboratory, New York City. Received for publication, October 28, 1925.

and amino-ethyl alcohol:



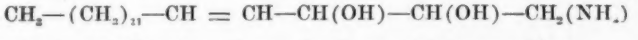
The best known phosphatid is lecithin to which the following formula is assigned:



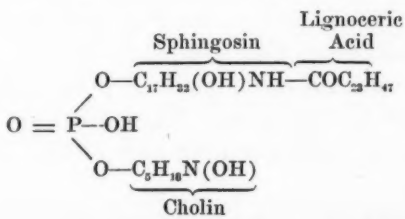
Thus we may regard lecithin as a fat in which one of the three fatty acids is substituted by phosphoric acid combined with the base cholin.

Cephalin resembles lecithin very much; it differs in possessing a different base, amino-ethyl alcohol instead of cholin, and possibly different fatty acids. According to recent workers[4] lecithin contains palmitic and oleic acids, while cephalin contains stearic and linoleic acids. The structure of cuorin is less understood; it resembles lecithin, and, according to Erlandsen[5] contains three fatty acids in the molecule, and a base, probably aminoethyl alcohol. Each of the three phosphatids described contains at least one fatty acid of the unsaturated variety. This makes them exceedingly liable to oxidation changes.

Sphingomyelin contains no unsaturated fatty acids and is, therefore, more stable. Its exact structure is not yet known. Levene[6] prepared what he thought was pure sphingomyelin and obtained the following products on hydrolysis: (1) phosphoric acid (no glycerin); (2) two fatty acids, one probably a hydroxy acid; (3) two bases: cholin and sphingosin. Sphingosin is said to have the following formula:



Levene suggests the following formula for sphingomyelin:




The phosphatids are generally classified according to the method introduced by Thudichum;[7] they are divided into groups depending upon the relation of nitrogen to phosphorus present in the molecule. Thus in lecithin and cephalin one atom of nitrogen and one of phosphorus occur; the N:P ratio is, therefore, 1:1. In sphingomyelin the N:P ratio is 2:1, while in cuorin the N:P ratio is 1:2. Thus lecithin and cephalin are monoaminomonophosphatids; cuorin is a monoaminodiphosphatid; while sphingomyelin is a diaminomonophosphatid.

The alcoholic extracts used as antigens in the Wassermann test consist of mixtures of the various phosphatids in which lecithin predominates by far, since it is the most abundant in the tissue and the most soluble in alcohol.

If the union between antigen and antibody is chemical in nature, then judging from the chemical structure of lecithin there are two ways possible by which a chemical union may take place between it and the positive serum: (1) at the double bonds of the unsaturated fatty acids; or (2) at the free hydroxyl groups present in the phosphoric acid and cholin in the lecithin molecule.

Noguchi's[8] statement that there seems to be a certain relationship between the iodine value and antigenic activity tends to indicate that the chemical union, if any, takes place at the double bond of the fatty acid in the lipoid molecule. To find out whether such is or is not the case, I saturated the double bond with hydrogen by the method of Paal and Oehme.[9] This method consists in shaking with hydrogen an alcoholic solution of lipoidal substance in the presence of a catalyzer, colloidal palladium.

## PROCEDURE

SATURATION OF DOUBLE BONDS.—*Preparation of Colloidal Palladium.*—Colloidal palladium was prepared according to the method of Kelber and Schwarz[10] which was somewhat modified. One gram of metallic palladium was heated with a few c.c. of concentrated HCl. The palladium dissolves with great difficulty in HCl, but on adding a few drops of con. $HNO_3$ it dissolves very readily, and a dark-red solution of palladium chloride is obtained. The free acid was evaporated off and the dry powder washed a few times with water and finally dissolved in 50 c.c. of water. One gram of gelatin was warmed in a small flask with 50 c.c. of water until it dissolved. The palladium chloride solution was made slightly alkaline with dilute ammonia and run into the gelatin slowly, drop by drop with constant stirring. Hydrazine-hydrate (50 per cent Kaulbaum) was then added from a burette drop by drop while stirring constantly. The liquid frothed, giving off gas, and became deep brownish-black. The frothing stopped, and the reduction was completed. The colloidal palladium was then dialyzed in a collodion bag against running water until the outside water gave no precipitate with $AgNO_3$, showing that all the chloride was removed. The palladium was then evaporated down to a small volume on the water-bath and, finally, in a vacuum, resulting in black lustrous scales.

The syphilitic antigen for this experiment was prepared at a low temperature. The muscle tissue of ten beef hearts was ground and spread in thin layers over sheets of cheesecloth stretched on wire frames and dried under the electric fan for thirty-six hours; at the end of this time it was completely dry. It was then ground to a fine powder. Five hundred grams of this powder was placed in a bag made of heavy filter-cloth. The mouth of the bag was supported by a rim of heavy wire which was held by four wires attached to a cork fitted in the wide neck of a twenty liter bottle. The top of the cork was placed a few millimeters below the upper end of the neck

of the bottle and was covered with mercury to make it air-tight. Three reflux condensers were placed in the cork. Two liters of ether were placed in the bottle. The lower end of the cloth-bag was about three inches above the surface of the ether. The bottle was then placed into a large pail of water which was kept warm over a small flame. The extraction with boiling ether was continued for forty-eight hours, at the end of that time the extraction was complete, since a subsequent extraction with fresh ether gave no coloration to the ether, and evaporation of 100 c.c. of the extracted ether left no deposit. The powder was then removed from the bag and dried in an incubator at 37° C. and extracted in the same cloth-bag with a liter of absolute alcohol in the same manner as the ether extraction, except that only one large size reflux condenser was used. This was attached to a vacuum pump and the boiling point of the alcohol thus kept between 35° and 37° C. This extraction was continued for forty-eight hours. The alcoholic extract was then evaporated to dryness in a vacuum for three weeks. A sticky brownish mass was obtained which weighed 46 grams.

Two grams of the dry substance was dissolved in 95 c.c. of alcohol to which was added one c.c. of glacial acetic acid. Two-tenths gram colloidal palladium was dissolved in 5 c.c. of water (it had to be warmed to be brought in solution). The colloidal palladium solution was then placed in a bottle, and the alcoholic lipoidal solution was added to it slowly, when a homogeneous dark suspension was formed. The bottle containing the mixture was then made air-tight and connected by a piece of soft rubber tubing to a reservoir filled with hydrogen under pressure. The hydrogen was prepared from zinc and sulphuric acid in a Kipp's apparatus, and the gas was passed through successive solutions of $KMnO_4$, $AgNO_3$, and KOH into a reservoir under water pressure. Great care had to be taken to remove all traces of arsenic and antimony from the materials used in the preparation of the hydrogen, for they destroy the catalytic action of the palladium. On leaving the reservoir the hydrogen was dried by passing through concentrated $H_2SO_4$ and anhydrous $CaCl_2$.

The bottle containing the lipoidal solution was then attached to a shaking apparatus connected to an electric motor and shaken for forty-eight hours with the hydrogen under pressure.

The colloidal mixture was then neutralized with ammonia, heated until the palladium coagulated, and filtered. The filtrate was then evaporated to dryness in a vacuum.

One gram of the hydrogenated lipoid was used for an iodine value determination to tell the degree of saturation. The rest was dissolved in 100 c.c. of alcohol and filtered; this was used for the antigenic titration.

A control was prepared by treating two grams of the lipoid in the same way, with the exception of leaving out the hydrogen.

The iodine number was determined by the Hübl method.[11] It showed a distinct reduction in the iodine value in the hydrogenated lipoid:

Iodine number of hydrogenated lipoid ___ 12
Iodine number of nonhydrogenated lipoid ___ 47.

While hydrogenation was not complete, yet enough saturation had taken place to impair seriously the antigenic property of the lipoid if the antigenic property was to depend upon the unsaturated bonds in the lipoidal molecule.

The antigenic titrations were done with an antisheep hemolytic system, one-fourth Wassermann quantities being used. Complement fixation was done in the ice box for four hours; sensitized red cells were then added, and the second incubation was done in the water-bath at 37° C. for twenty minutes and the results read.

The antigens were made up in two dilutions: 1:10 and 1:40 by slowly adding saline from a pipette to a measured amount of antigen into a flask, shaking it all the while.

A series of 13 test tubes were set up; the first 4 tubes receiving increasing amounts of the 1:40 diluted antigen, and the remaining tubes receiving increasing amounts of the 1:10 diluted antigen.

The first 6 tubes contained 0.05 c.c. syphilitic serum each, thus serving as a test for the antigenic power of the antigen. Tubes 7 to 11 inclusive contained no serum (except tube 7 which contained 0.1 c.c. of normal serum and served to test the specificity of the antigen) and demonstrated the anticomplementary property. The last two tubes contained no serum and no complement and showed the hemolytic power of the antigen.

The following signs were used in writing down the results:

++++ = complete inhibition of hemolysis
+++ = slight hemolysis
++ = half hemolysis
+ = slight inhibition
0 = complete hemolysis.

The titration in the following table shows that the binding power of an antigen used in the complement-fixation test for syphilis is not destroyed by saturating the double bonds in the unsaturated fatty acids in the lipoid molecule.

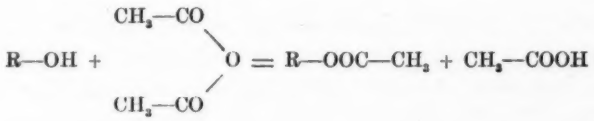
TABLE I

TITRATION OF SATURATED ANTIGEN

| ANTIGENIC DILUTION | 1-40 | | | | 1-10 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PROPERTIES | ANTIGENIC | | | | | | ANTICOMPLEMENTARY | | | | | HEMOLYTIC | |
| Tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Ant. c.c. | .01 | .02 | .05 | 0.1 | .05 | 0.1 | 0.2 | 0.5 | .75 | 1.0 | 1.2 | 0.5 | 1.0 |
| Saturated Ant. | 0 | 0 | 0 | ++ | ++++ | ++++ | 0 | 0 | 0 | + | ++ | ++++ | +++ |
| Control | 0 | 0 | 0 | +++ | ++++ | ++++ | 0 | 0 | 0 | ++ | +++ | ++++ | ++++ |

SUBSTITUTION OF HYDROXYL GROUPS

The next step was to find out if the OH groups in the lipoids were responsible for the binding power of the antigen. The most convenient method to accomplish this is to block off the OH groups by introducing acetyl radicles in their place. This may be done by treating a lipoid with acetic anhydrid. The following reaction takes place:



PROCEDURE

Four grams of the lipoidal substance prepared in the previous experiment were heated for two hours under a reflux condenser with 10 c.c. of freshly distilled acetic anhydrid. The acetylated lipoid was then emulsified with about 30 c.c. of water and shaken up with an excess of acetone in which the lipoid precipitated. The precipitate was then washed with fresh portions of acetone a few times. It was then emulsified with about 30 c.c. of water and dialyzed in a parchment bag against running water for forty-eight hours, thus removing all the acetic acid and the anhydrid. The lipoidal material was then precipitated with acetone and dried in a vacuum for two weeks.

One gram of the dry substance was dissolved in 50 c.c. of alcohol and titrated for antigenic properties as in the previous experiment.

As a control four grams of the lipoidal substance prepared in the previous experiment were treated similarly except that the acetylation was left out. One gram of the dry substance was dissolved in 50 c.c. of alcohol.

As the titration in the table below shows, the substitution of $CH_3COO$ groups for the OH groups in the lipoidal molecules does not destroy their antigenic properties.

TABLE II

ACETYLATION OF LIPOID

| ANTIGENIC DILUTION | 1-40 | | | | 1-10 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PROPERTIES | ANTIGENIC | | | | | | ANTICOMPLEMENTARY | | | | | HEMOLYTIC | |
| Tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Ant. c.c. | .01 | 0.2 | .05 | 0.1 | .05 | 0.1 | 0.2 | 0.5 | .75 | 1.0 | 1.2 | 0.5 | 1.0 |
| Acetylated Ant. | 0 | 0 | 0 | + | ++++ | ++++ | 0 | 0 | + | ++ | ++++ | ++++ | ++++ |
| Control | 0 | 0 | 0 | +++ | ++++ | ++++ | 0 | 0 | 0 | + | ++++ | ++++ | ++++ |

SUMMARY

1. Saturation of the double bonds in the fatty acids of the lipoidal antigen used for complement-fixation test in the diagnosis of syphilis, does not materially interfere with its antigenic properties; hence the unsaturated fatty acids in the lipoid molecule are not responsible for the specificity of the Wassermann reaction.

2. Blocking off the hydroxyl groups in the lipoid molecule by substituting acetyl groups does not materially impair the antigenic value of the lipoid.

Since chemical union would most likely take place either at the double bonds of the unsaturated fatty acids or at the hydroxyl groups, and the blocking of both of these possibilities does not materially diminish the specific value of the antigen, it follows that the Wassermann reaction is probably not based upon a chemical reaction, but rather that physical phenomena are involved in it.

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## THE SIGNIFICANCE OF BLOOD CULTURES*

By Theodore C. Lawson M.D., New York, N. Y.

As IT was thought that the large clinical and pathologic facilities at Bellevue Hospital would afford opportunities for studying the general value of taking blood cultures from the diagnostic, therapeutic and prognostic standpoints, an attempt has been made in this study to tabulate and follow the results of all blood cultures taken between July 1, 1922, and July 1, 1924.

In this paper, the following aspects are covered: The total number of blood cultures taken, with the diseases for which they were taken; the total number of positive cultures; the diseases for which these were positive, with the specific number of each and per cent of positive cultures as compared to the total number of blood cultures for each disease; total deaths for each disease; the organisms isolated for each disease; the total deaths for the organism isolated; and the end-results or the percentage of deaths in relation to positive blood cultures. The object of this paper is to record the results of blood culturing as a whole, not to follow up any one definite disease, organism, or series of cases for a comprehensive and detailed review.

During the above-stated period, there was a total of 2,092 recorded blood cultures. The diseases for which these were taken include a total of 105 different medical and surgical conditions. It should be stated here that the diagnoses recorded at the time the cultures were taken were by no means final in all cases. The culture was taken, in many instances, as an aid in the establishment of a final diagnosis. Thus, many of the cases recorded as typhoid or malaria were followed by question marks, and the diagnosis stated was the most plausible tentative one at that time. Again, it will be seen that many of the diagnoses recorded appear at first to be absurd, namely, some of the anemias, nephritis, tabes dorsalis, alcoholism, etc. These were the admitting diagnoses, but the patients subsequently developed some definite superimposed complication, with temperature, etc., which warranted taking a culture. At the head of the list of diseases for which blood cultures were taken, is typhoid fever, with 357 blood cultures; next, general sepsis,

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with 226 (not including postabortal and postpartum sepsis); lobar pneumonia, with 200; acute endocarditis, with 169; postpartum sepsis, 91; subacute bacterial endocarditis, 51; and so on. Chart 1 shows a list of all these diseases with the total number of blood cultures taken during each of the two years, with a total for the two years.

The total number of recorded *positive* cultures obtained during this time for some of the more important diseases are:

*Typhoid.*—Forty-one cases, or 11.2 per cent of the total cultures taken for this disease. Widals were positive in 77 cases, so that there were 53.2 per cent of positive typhoid cultures to total positive Widals.

*General sepsis.*—Thirty-nine cases, or 17.7 per cent of all cultures taken for this disease.

*Lobar pneumonia.*—Forty-two cases, or 21 per cent.

*Acute endocarditis.*—Sixteen cases or 10 per cent.

*Cerebrospinal meningitis.*—Fifteen cases or 45.4 per cent.

*Osteomyelitis.*—Seven cases or 16.4 per cent.

*Postpartum sepsis.*—Seven cases or 7.7 per cent.

And so on for several more or less important diseases, culturally speaking. This data is tabulated in toto on Chart 1 in columns 4 and 5. The total number of positive cultures for all diseases is 221, or 10.5 per cent of the total cultures taken. In many cases more than one positive blood culture was taken, and in these circumstances only one was used for our purposes.

It is interesting to note the number of recorded deaths for each disease and to tabulate the percentage of deaths in relation to the positive blood cultures for each disease, together with the per cent of deaths to the total cases for each disease. Thus, in typhoid fever, during this period of time, there was a total of 11 recorded deaths, or 27 per cent of the total of 41 cases with positive blood cultures, with a percentage of deaths in relation to a total cases of only 3 per cent. Even though all of the 357 cases were not typhoid fever, yet it must be granted that this percentage is remarkably low. Sepsis claimed 31 cases, or 79.5 per cent of positive blood cultures, a rather high percentage, but yet the high fatality of general septicemia, caused for the most part by the streptococci and the staphylococci, as is true in these cases, is a notorious fact. Still, this is 13.7 per cent of the total blood cultures taken for suspected sepsis, a moderately low figure. Of the 42 cases of lobar pneumonia with positive blood cultures, the majority of which were due to pneumonia, 34 died, or 81 per cent. Of course, this strikes one as being a very high figure for percentage of deaths in pneumonia, but when one realizes that these are all pneumonias with positive blood cultures and that it represents deaths which were only 17 per cent of the total 200 cases of lobar pneumonia for which blood cultures were taken, one realizes that it is not so high a mortality rate as might at first be supposed. One cannot compare this 17 per cent of deaths to the average mortality rate, which is approximately 30 per cent of deaths for all cases of pneumonia, because of the fact that all the cases of lobar pneumonia were by no means cultured, and this does not represent any definite proportion of the total pneumonias admitted to the wards.

CHART 1

BLOOD CULTURES AT BELLEVUE HOSPITAL FROM JULY 1, 1921 TO JULY 1, 1923

| DISEASES | 1<br>1921-22 | 2<br>1922-23 | 3<br>TOTAL FOR BOTH YEARS | 4<br>NUMBER OF POSITIVE | 5<br>PER CENT POSITIVE OF TOTAL TAKEN | 6<br>NUMBER OF DEATHS | 7<br>PER CENT DEATHS TO TOTAL POSITIVES | 8<br>PER CENT DEATHS TO TOTAL CULTURES TAKEN |
|---|---|---|---|---|---|---|---|---|
| 1. Typhoid | 194 | 163 | 357 | 41 | 11.2 | 11 | 27. | 3. |
| 2. Widals, positive | 37 | 40 | 77 | – | 53.2 of positive cultures to pos. Widals. | | | |
| 3. Sepsis, general | 137 | 89 | 226 | 39 | 17.7 | 31 | 79.5 | 13.7 |
| 4. Sepsis, postabortal | 13 | 34 | 47 | 2 | 4.2 | 2 | 100. | 42.5 |
| 5. Sepsis, postpartum | 73 | 18 | 91 | 7 | 7.7 | 3 | 43. | 3.3 |
| 6. Endocarditis, subac. bact. | – | 51 | 51 | 7 | 13.7 | 4 | 57. | 8. |
| 7. Endocarditis, acute | 125 | 44 | 169 | 16 | 10. | 13 | 81.2 | 7.6 |
| 8. Endocarditis, rheumatic | – | 9 | 9 | – | – | – | – | – |
| 9. Pericarditis, acute | 2 | 5 | 7 | – | – | – | – | – |
| 10. Myocarditis, chronic | 6 | – | 6 | – | – | – | – | – |
| 11. Chr. cardiovalvular disease | 17 | 31 | 48 | – | – | – | – | – |
| 12. Pneumonia, lobar | 123 | 77 | 200 | 42 | 21. | 34 | 81. | 17. |
| 13. Pneumonia, broncho | 3 | 6 | 9 | – | – | – | – | – |
| 14. Meningitis, cerebrospinal | 17 | 16 | 33 | 15 | 45.4 | 11 | 73.3 | 33.3 |
| 15. Arthritis, infectious | 17 | 12 | 29 | 4 | 13.8 | 1 | 25. | 3.4 |
| 16. Arthritis, gonococcal | 1 | 2 | 3 | – | – | – | – | – |
| 17. Osteomyelitis | 25 | 18 | 43 | 7 | 16.4 | 3 | 43. | 7. |
| 18. Tuberculosis, in general | 9 | 9 | 18 | – | – | – | – | – |
| 19. Anemias | 5 | 9 | 14 | – | – | – | – | – |
| 20. Purpuras | 4 | 5 | 9 | – | – | – | – | – |
| 21. Leucemias | 6 | – | 6 | – | – | – | – | – |
| 22. Fractures | 6 | 6 | 12 | – | – | – | – | – |
| 23. Abscesses | 23 | 24 | 47 | 2 | 43. | – | – | – |

CHART 1—CONT'D

| DISEASES | 1<br>1921-22 | 2<br>1922-23 | 3<br>TOTAL FOR BOTH YEARS | 4<br>NUMBER OF POSITIVE | 5<br>PER CENT POSITIVE OF TOTAL TAKEN | 6<br>NUMBER OF DEATHS | 7<br>PER CENT DEATHS TO TOTAL POSITIVES | 8<br>PER CENT DEATHS TO TOTAL CULTURES TAKEN |
|---|---|---|---|---|---|---|---|---|
| 24. Mastoiditis | 13 | 17 | 30 | 3 | 10. | 1 | 33.3 | 3.3 |
| 25. Erysipelas | 2 | 5 | 7 | 4 | 57. | 4 | 100. | 57. |
| 26. Pelvic infection | 1 | 1 | 2 | 1 | 50. | 1 | 50. | 100. |
| 27. Cellulitis | 10 | 7 | 17 | 2 | 11.8 | 2 | 100. | 11.7 |
| 28. Anthrax | 7 | 4 | 11 | 3 | 27.2 | 2 | 66.6 | 18.2 |
| 29. Influenza | 3 | 3 | 6 | 1 | 16.6 | 1 | 100. | 16.6 |
| 30. Pemphigus | – | 4 | 4 | 1 | 25. | 1 | 100. | 25. |
| 31. Peritonitis | 5 | 2 | 7 | 2 | 29.5 | 2 | 100. | 28.5 |
| 32. Empyema | 10 | 2 | 12 | 2 | 16.6 | 1 | 50. | 8.3 |
| 33. Carbuncle | 3 | 3 | 6 | 1 | 16.6 | 1 | 100. | 16.6 |
| 34. Acute rheumatic fever | 14 | 9 | 23 | 3 | 39. | 3 | 100. | 13. |
| 35. Infected hand | – | 2 | 2 | 1 | 50. | 1 | 100. | 50. |
| 36. Aneurysm | 1 | 1 | 6 | 1 | 50. | 1 | 100. | 100. |
| 37. Fever | 5 | 1 | 6 | 1 | 16.6 | 1 | 100. | 100. |
| 38. Phlebitis | 5 | – | 5 | 1 | 20. | 1 | 100. | 20. |
| 39. Paratyphoid fever | 3 | – | 3 | 1 | 33.3 | – | – | – |
| 40. Raynaud's disease | 2 | – | 2 | 1 | 50. | 1 | 100. | 50. |
| 41. Sinus thrombosis | 17 | 2 | 19 | 2 | 10.5 | – | – | – |
| 42. Pleurisy | 3 | 4 | 7 | – | – | – | – | – |
| 43. Thrombophlebitis | 2 | 6 | 8 | – | – | – | – | – |
| 44. Pyelitis | 7 | 3 | 10 | – | – | – | – | – |
| 45. Encephalitis | 8 | 7 | 15 | – | – | – | – | – |
| 46. Undiagnosed | 60 | 39 | 99 | – | – | – | – | – |
| 47. Not stated | 50 | 75 | 125 | – | – | – | – | – |
| 48. Miscellaneous | 75 | 82 | 157 | – | – | – | – | – |
| Total or average | | | 2092 | 221 | 10.5 | 137 | 62.2 | 6.54 |

**Deaths in Number of Days After Last Positive Culture, with Percent of Deaths of Each to Total Deaths.**

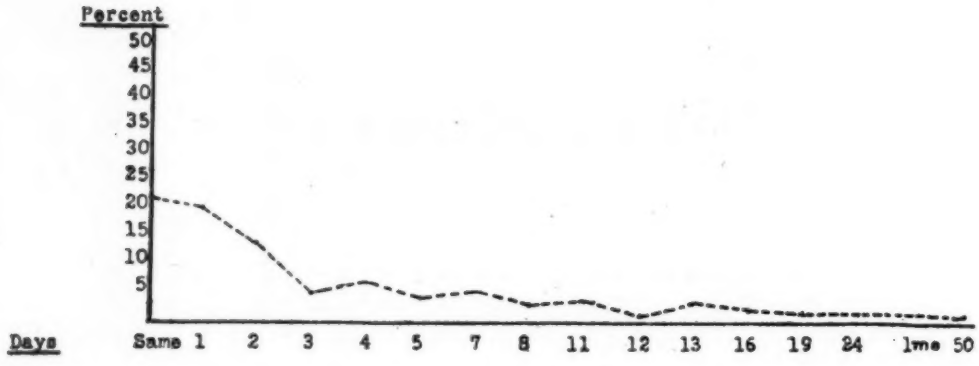



Chart 1.—Continued.

Of the 16 cases of acute endocarditis, 13 died, or 81.2 per cent. It is a well-known fact that acute or malignant endocarditis is highly fatal, so this high figure is not surprising. The percentage of deaths in relation to the total 169 cases of acute endocarditis for which cultures were taken is 7.6, a fact in itself which assures one that these cases of endocarditis either were not in their acute stage or were not endocarditis at all.

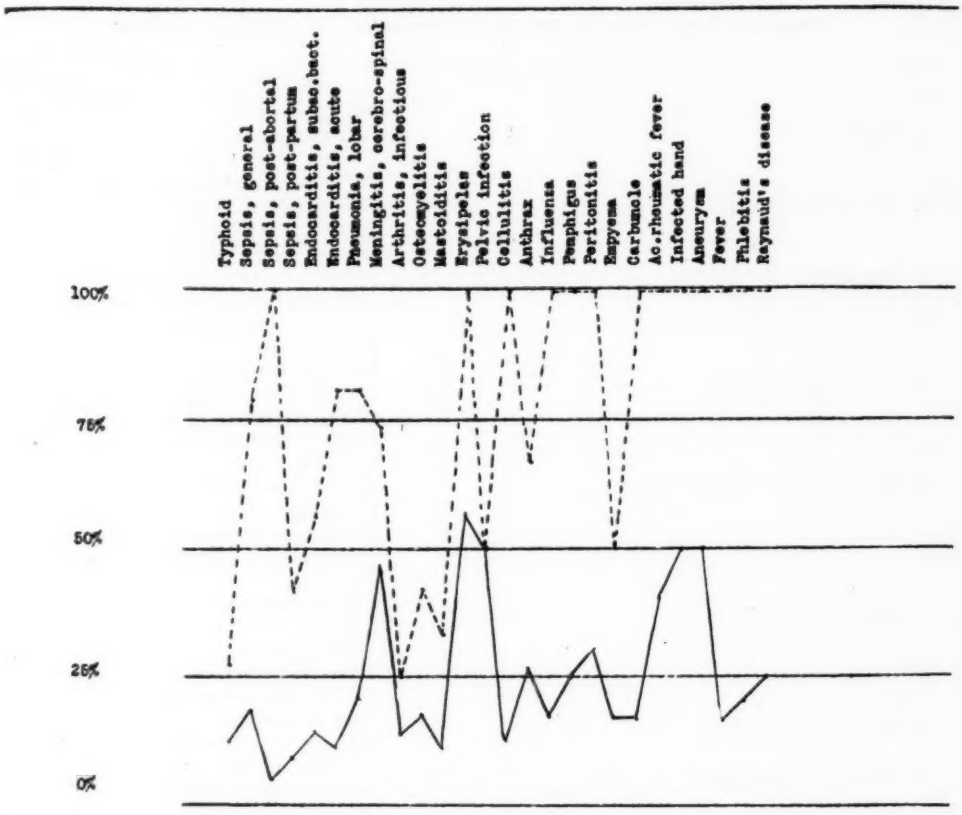
Eleven of the 15 cases with cerebrospinal meningitis died, or 73.3 per cent. This amounts to 33.3 per cent of the total 33 cases diagnosed as such. Both these figures are high, due mainly to the fact that over half (8) of these cases with positive blood cultures had pneumococci isolated; 5 had Staphylococcus aureus, while one each had meningococci and Streptococcus hemolyticus. It is fair to assume that those cases of meningitis with pneumococcus septicemia had pneumococcus meningitis, and the high mortality rate of this is well known. One outstanding fact is that, in this series of cases, only one had meningococcus septicemia. It is probable that the blood culture here was taken early in the disease when a meningococcus septicemia exists in Meningococcus meningitides.

There were 3 deaths of the 7 cases of osteomyelitis with positive blood cultures, or 43 per cent, making a death rate of only 7 per cent of the total 43 cases with osteomyelitis. This is as small a number as can be expected, especially in view of a constant focus of blood infection, which an osteomyelitis affords.

Of the 7 cases of erysipelas that were cultured, 4 were positive, and all four cases died, giving a 100 per cent mortality of the positive cultures, or a mortality of 57 per cent of all that were cultured. Two of these had Streptococcus hemolyticus, one streptococcus, and the fourth, Staphylococcus aureus.

CHART NO. 2.

Percent Positives to Total Cultures and Percent Deaths to Total Positives.




——— = Percent positive cultures to total cultures taken.

------ = Percent deaths to total positive cultures.

There were 11 cases of anthrax that were cultured, 3 of which were positive, or 27.2 per cent. Two of these died, giving a mortality of the total cultured of 18.2 per cent.

It is deemed unnecessary to enumerate the number of deaths with percentages of the other diseases, the results in tabulation being found on Chart 1, Columns 6, 7, and 8.

The proportion of deaths from all cases of septicemia in total positive blood cultures was 62.2 per cent. The proportion of known deaths from septicemia to total blood cultures taken was 6.54 per cent.

Generally speaking, it may be said that a large number of blood cultures are necessary and justifiable in certain diseases, especially as an aid to diagnosis. This is true in most diseases which yield a comparatively high percentage of positive blood cultures in relation to the total taken, and may be said to include general sepsis, lobar pneumonia, cerebrospinal meningitis, erysipelas and others. There is the additional fact, however, that the number

CHART 3

DISEASES WITH POSITIVE BLOOD CULTURES AND ORGANISMS ISOLATED

| DISEASES | NO. CASES | ORGANISMS | NO. CASES |
|---|---|---|---|
| 1. Typhoid | 41 | B. typhosus | 38 |
| | | Streptococcus | 2 |
| | | Staph. aureus | 1 |
| 2. Infectious arthritis | 4 | Strept. viridans | 1 |
| | | Staph. hemolyticus | 2 |
| | | Strept. hemolyticus | 1 |
| 3. Erysipelas | 4 | Strept. hemolyticus | 2 |
| | | Staph. aureus | 1 |
| | | Streptococcus | 1 |
| 4. Acute endocarditis | 16 | Strept. hemolyticus | 4 |
| | | Gonococcus | 1 |
| | | Strept. viridans | 6 |
| | | Staph. hemolyticus | 1 |
| | | Pneumococcus | 2 |
| | | Staph. aureus | 2 |
| 5. Subac. bact. endocarditis | 7 | Streptococcus | 1 |
| | | Strept. viridans | 5 |
| | | Meningococcus | 1 |
| 6. Sepsis, general | 39 | Staph. aureus | 14 |
| | | Strept. hemolyticus | 11 |
| | | B. coli | 5 |
| | | Streptococcus | 1 |
| | | Strept. viridans | 1 |
| | | Staph. albus | 2 |
| | | Pneumococcus | 4 |
| | | Colon-typhoid group | 1 |
| 7. Sepsis, postabortal | 2 | Streptococcus | 1 |
| | | Strept. hemolyticus | 1 |
| 8. Sepsis, postpartum | 7 | Staph. aureus | 1 |
| | | Strept. hemolyticus | 6 |
| 9. Pneumonia, lobar | 42 | Pneumococcus | 33 |
| | | Strept. hemolyticus | 5 |
| | | Staph. aureus | 3 |
| | | Strept. viridans | 1 |
| 10. Meningitis, cerebrospinal | 15 | Staph. aureus | 5 |
| | | Pneumococcus | 8 |
| | | Strept. hemolyticus | 1 |
| | | Meningococcus | 1 |
| 11. Osteomyelitis | 7 | Staph. aureus | 4 |
| | | Strept. hemolyticus | 2 |
| | | Staph. albus | 1 |
| 12. Acute rheumatic fever | 3 | Staph. aureus | 2 |
| | | Strept. viridans | 1 |
| 13. Cellulitis | 2 | Staph. aureus | 2 |
| 14. Anthrax | 2 | B. anthracis | 2 |
| 15. Aneurysm | 1 | Strept. hemolyticus | 1 |
| 16. Phlebitis | 1 | Strept. hemolyticus | 1 |
| 17. Fever | 1 | Strept. hemolyticus | 1 |
| 18. Carbuncle | 1 | Staph. aureus | 1 |
| 19. Empyema | 2 | Staph. albus | 1 |
| | | Pneumococcus | 1 |
| 20. Sinus thrombosis | 2 | Staph. albus | 1 |
| | | Strept. hemolyticus | 1 |
| 21. Pelvic inflammation | 1 | Streptococcus | 1 |
| 22. Raynaud's disease | 1 | Strept. hemolyticus | 1 |
| 23. Mastoiditis | 3 | Pneumococcus | 2 |
| | | Strept. hemolyticus | 1 |

CHART 3—CONT'D

| DISEASES | NO. CASES | ORGANISMS | NO. CASES |
|---|---|---|---|
| 24. Peritonitis | 2 | Pneumococcus | 1 |
| | | Strept. hemolyticus | 1 |
| 25. Pemphigus | 1 | Staph. aureus | 1 |
| 26. Abscess | 2 | Strept. hemolyticus | 1 |
| | | Staph. aureus | 1 |
| 27. Influenza | 1 | Streptococcus | 1 |
| 28. Paratyphoid fever | 1 | B. paratyphosus | 1 |

CHART 4

ORGANISMS ISOLATED WITH DEATHS AND PERCENTAGE DEATHS FOR EACH

| | NO. CASES | NO. DEATHS | PER CENT DEATHS TO CASES |
|---|---|---|---|
| 1. Pneumococcus | 51 | 38 | 72.5 |
| 2. Streptococcus hemolyticus | 42 | 26 | 61.9 |
| 3. B. typhosus | 38 | 11 | 28.9 |
| 4. Staphylococcus aureus | 38 | 31 | 80. |
| 5. Streptococcus viridans | 15 | 10 | 66.6 |
| 6. Streptococcus | 8 | 8 | 100. |
| 7. B. coli | 5 | 4 | 80. |
| 8. Staphylococcus albus | 5 | 3 | 60. |
| 9. Staphylococcus hemolyticus | 2 | 2 | 100. |
| 10. Meningococcus | 2 | 1 | 50. |
| 11. B. anthracis | 3 | 2 | 66.6 |
| 12. Colon-typhoid group | 1 | 0 | – |
| 13. Gonococcus | 1 | 1 | 100. |
| 14. B. paratyphosus | 1 | 0 | – |
| Total | 221 | 137 | 72.2 (average per cent) |

CHART 5

DEATHS IN NUMBER OF DAYS AFTER LAST POSITIVE BLOOD CULTURE

| | NO. OF DAYS | NO. OF CASES | PER CENT TO TOTAL DEATHS |
|---|---|---|---|
| 1. | Same day | 31 | 22.6 |
| 2. | 1 day | 29 | 21.1 |
| 3. | 2 days | 20 | 14.6 |
| 4. | 3 " | 7 | 5.1 |
| 5. | 4 " | 10 | 7.3 |
| 6. | 5 " | 6 | 4.3 |
| 7. | 7 " | 7 | 5.1 |
| 8. | 8 " | 4 | 2.9 |
| 9. | 11 " | 5 | 3.6 |
| 10. | 12 " | 1 | .7 |
| 11. | 13 " | 4 | 2.9 |
| 12. | 16 " | 3 | 2.1 |
| 13. | 19 " | 3 | 2.1 |
| 14. | 24 " | 3 | 2.1 |
| 15. | 1 month | 3 | 2.1 |
| 16. | 50 days | 1 | .7 |
| | Total | 137 | |

of unnecessary and sterile blood cultures can be lessened, particularly from a diagnostic point of view, in many diseases yielding a low percentage of positives in relation to the total taken, such as typhoid, postabortal sepsis, postpartum sepsis, acute endocarditis, acute rheumatic fever, mastoiditis, etc., and others in which no positive blood cultures were obtained at all, such as chronic cardiovalvular disease, the tuberculous affections, fractures, empyema.

It may be said that if it is thought that a possible positive blood culture will aid in the establishment of a known diagnosis from an unknown, that it is justifiable, whatever the circumstances. Chart 2 shows graphically the percentage of positive cultures in relation to total cultures, and the percentage of deaths in relation to total positive cultures.

The diseases in which positive blood cultures were obtained totaled 28, or 26.6 per cent of the total number of conditions for which they were taken. These diseases with positive blood cultures with organisms isolated for each disease and percentage of each organism in relation to the total for the disease will be found on Chart 3.

It is interesting to study the end-results of septicemias according to the organisms isolated from blood cultures. There was a total of 51 cases with cultures positive for pneumococci; of which 38 or 72.5 per cent died. Most of these had lobar pneumonia. It has been seen that the percentage of deaths in relation to positive cultures in this disease was 81 per cent, so that the figures for the pneumococcus septicemias are somewhat better as far as end-results are concerned. There were 42 cases of positive cultures of Streptococcus hemolyticus. Of these, 26 or 61.9 per cent died. The high death rate of septicemia from this organism is well known, and it can be safely said that this is about as low a percentage for Streptococcus hemolyticus septicemia as can be found. It is evident that transfusions and blood antiseptic solutions are still entirely ineffective in the majority of instances. Of the 38 cases of typhoid with positive cultures of B. typhosus, 11 or 28.9 per cent died. There were 38 Staphylococcus aureus septicemias, with 31 deaths or 80 per cent—a high figure, as is expected. Most of these for any one disease occurred in general sepsis, where 14 cases had Staphylococcus aureus septicemia. Fifteen cases had Streptococcus viridans septicemia, with death claiming 10 of these, or a high mortality of 66.6 per cent. There were 8 cases of positive streptococcus blood cultures, with 8 deaths, or 100 per cent mortality. A high rate is to be expected, but one as high as this seems to be too high, though it may be mentioned that if more cases of streptococcus septicemia were included, some cases would undoubtedly have been found with recovery. The remainder of the organisms isolated with number of deaths and percentage of deaths in relation to positive cultures are to be found in Chart 4.

A tabulation has been made ascertaining the number of days that elapsed from the date of the initial positive blood culture to the date of death or discharge from the hospital. Of course, this is not so valuable in the cases that were discharged, for in many cases the patients were discharged unimproved against the orders of the physician in charge, and may have died at home, so that they cannot be brought into our records. Even in the cases with positive blood cultures, the number of days following a positive culture that elapsed before death occurred does not necessarily indicate the seriousness of the disease, as a patient might have had a septicemia for days before being brought into the hospital and may have died soon after the positive culture was obtained.

Of the 221 cases with positive blood cultures, there were 137 known deaths, or 62.2 per cent of the total positive cultures. The greater number of deaths for any one day occurred on the same day that the positive culture was taken, less on the day after, and so on. Thirty-one died on the same day as the positive culture was taken, 29 on the day after, 20 two days after, 7 three days after, 10 four days after, 6 five days after, etc., the number of deaths being in inverse ratio to the number of days after the last positive culture was taken. The number of days after positive culture that death occurred is found on Chart 5.

## SUMMARY

1. A total of 2,092 recorded blood cultures, taken during two years, was studied and the final end-results tabulated. There were 105 different medical and surgical conditions for which blood cultures were taken. Many cultures were taken to aid in the establishment of a diagnosis. The greatest number of cultures were taken from patients of suspected typhoid fever (357 cultures), with a small progressive number from cases of sepsis, lobar pneumonia, acute endocarditis and others.

2. Altogether 221 positive blood cultures were obtained (10.5 per cent of the total cultures taken).

3. The proportion of deaths from all cases of septicemia to total positive blood cultures was 62.2 per cent. The proportion of known deaths from septicemia to total blood cultures taken was 6.54 per cent.

4. The diseases with positive blood cultures totalled 28, or 26.6 per cent of the total number of conditions for which they were taken.

5. The organisms isolated include the usual pathogenic ones, the majority of positive cultures being composed of pneumococci, Staphylococcus aureus, B. typhosus, Streptococcus hemolyticus and Streptococcus viridans.

6. The percentage of deaths in relation to total positive cultures for each organism range from 100 per cent with gonococci, Staphylococcus hemolyticus and streptococci to 61.9 per cent for Streptococcus hemolyticus, 50 per cent for meningococci, and 28.9 per cent for B. typhosus. The average percentage of deaths in relation to total positive cultures for all organisms was 72.2 per cent.

7. The majority of deaths occurred on the same day or within four days after the first blood culture was taken.

# A LABORATORY STUDY OF AN EXTENSIVE EPIDEMIC OF SEPTIC SORE THROAT*

By Marion B. Coleman, B.S., and Mary W. Wheeler, M.A.
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IN EPIDEMICS of so-called septic sore throat which have been investigated previously, a hemolytic streptococcus has generally been considered the etiologic agent. Such reports are so numerous and well known that a review of the literature is unnecessary. An epidemic of 500 cases of septic sore throat occurring in a village of approximately 3,500 inhabitants during May and June of 1925† offered an opportunity to apply the methods now available for investigating such infections. It was thought of interest, moreover, to determine the relationship of the hemolytic streptococci present to those isolated from other infections, especially those from scarlet fever, and also to exclude the possible presence of hitherto unsuspected bacterial incitants.

The clinical aspect of the acute cases resembled that described by other workers. The onset was usually sudden with headache, chills, muscular soreness, and diffuse redness and swelling of the throat. In some cases, the cervical glands were swollen and tender. No rash was noted in any case, and no desquamation was reported.

An intensive bacteriologic examination was made of a few of the acute cases. Swabbings from the throats were cultured directly on various kinds of media, animals were inoculated, and preparations were made for microscopic examination. The blood was cultured in two instances, but no growth of bacteria was obtained. The microscopic and cultural examinations of the throat swabbings showed hemolytic streptococci to be the predominating organisms. Rabbits inoculated intrapleurally with throat swabbings from one of the patients whose blood was cultured and from one other acute case, died after six and sixteen days, respectively. Pure cultures of hemolytic streptococci were recovered from their blood and from pleural abscesses. Rabbits inoculated intravenously with 0.5 c.c. and 1 c.c. of broth cultures of the streptococci recovered from the first rabbits, failed to show any signs of infection. These cultures proved fatal for mice in 0.2 c.c. amounts, but the virulence was not increased by passage through 16 of these animals. In an attempt to rule out the possibility of infection with *B. pneumosintes,* 2 rabbits were inoculated intratracheally with the same throat swabbings as those used for the intrapleural inoculations. These animals showed no abnormalities in blood counts, nor any evidence of a pneumosintes infection when killed forty-eight hours later. Further passage by means of intratracheal inoculation of lung tissue failed to induce any lesions in rabbits.

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

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†An epidemiologic study of the outbreak was made by Dr. B. E. Roberts, Epidemiologist of the State Department of Health, whose report will be published separately.

Since the clinical aspect of septic sore throat is not unlike that of scarlet fever, it was thought advisable to determine the relation of the streptococci isolated from these cases to streptococci from scarlet fever by studying the toxin production and serologic reactions of a number of strains.

### TOXIN PRODUCTION

Culture filtrates of 12 strains isolated from acute convalescent and recovered cases were tested intracutaneously on goats which were known to be susceptible to a standard scarlet fever streptococcus toxin. Under the conditions of the test, 6 of the strains produced no toxin. Five other strains produced toxins of very low potency, 4 of which were neutralized by an immune goat serum produced against the standard strain of scarlet fever streptococcus, while the fifth toxin was not completely neutralized in the dilutions tested. In the case of 2 strains, successive passage through 15 mice failed to increase their virulence or toxicity.

One strain isolated from a recovered case produced a potent toxin neutralized by the immune goat serum. This patient had no previous history of scarlet fever. His serum neutralized the toxin produced by the strain isolated from his throat and also the standard scarlet fever streptococcus toxin. The patient did not react to the standard scarlet fever streptococcus toxin; but the significance of this result is doubtful, since no test had been made previously.

During July and August the intracutaneous test for susceptibility to scarlet fever streptococcus toxin was made on a group of 20 persons who had had definite cases of septic sore throat during the epidemic, although cultures had been submitted from only 6 of them. It was thought that, if an unusually large proportion of persons failed to react to the toxin, it would indicate that this immunity might have been acquired as a result of the recent infection with streptococci. In this small series the findings were not suggestive, since 30 per cent of those tested reacted definitely to the toxin, which is approximately the same percentage as that found in a group of normal individuals of the same ages (eight to fifty years).

### AGGLUTINATION AND PRECIPITIN TESTS

Seven of the strains were tested for agglutination in rabbit serums immune to the Dochez strain of scarlet fever streptococcus. Homogeneous suspensions were obtained for these tests by transferring the cultures daily for two weeks in pneumococcus broth. The tests were made in dilutions ranging from 1:40 through 1:1280 (the titer of the serum) and were read after being incubated for two hours at 37° C. and again after standing overnight in the refrigerator. Five of these strains were agglutinated in the 1:1280 dilution of the serum, one giving a much less definite reaction than the others. The other 2 strains tested were agglutinated in the 1:80 and 1:160 dilutions of the serum, respectively.

An extract prepared by heating the sediment from an eight-day broth culture of the Dochez strain of scarlet fever streptococcus, gave a definite precipitin reaction with the homologous immune rabbit serums used for the

agglutination tests. Precipitinogens prepared in the same way from 4 of the strains of streptococci, 1 being the toxin producer, gave equally definite reactions with these serums.

## SUMMARY

Hemolytic streptococci were found to be the predominating organisms in throat cultures from the acute cases studied in an extensive epidemic of septic sore throat. The toxicity of 12 strains was tested. One produced a potent toxin neutralized by our standard scarlet fever antistreptococcus serum and also by the patient's serum. The other 11 produced little or no toxin. The precipitinogens and agglutinating antigens prepared from 4 of the nontoxic strains could not be distinguished from those prepared from the 1 toxic strain nor from the Dochez strain of scarlet fever streptococcus.

Among a group of 20 convalescents, 30 per cent gave definite skin reactions to a standard scarlet fever streptococcus toxin, which is approximately the same proportion as in a group of normal individuals of the same age (eight to fifty years).

# *LABORATORY METHODS*

## A SIMPLE METHOD FOR CULTIVATING ANAEROBES BY MEANS OF PHOSPHORUS*

By P. L. Varney, M.S., St. Louis, Mo.

DURING a course of study of anaerobic mouth organisms, it became necessary to devise some method whereby large numbers of tube and plate cultures of these organisms could be quickly and economically placed under strictly anaerobic conditions with no danger of contamination of the cultures at any stage of the process.

Numerous methods for anaerobic tube and plate cultures are to be found in the literature, but they all have some objection because of which they have been discarded for a better method. With certain of these methods, the apparatus is left in a sticky, blackened condition, and an undue amount of labor is necessitated when large numbers of cultures must be incubated and examined. The various pyrogallate methods, including that of Wright's, which had been used previously in this work, are open to this objection. Others require expensive and complicated apparatus or chemicals, are not certain in action, require constant watching, are dangerous to use, or allow possible contamination during incubation or removal of the plates or tubes from the apparatus.

The simplest and best of the various methods are those which depend on the removal of oxygen, by reduction or displacement, from large containers in which the inoculated material has been placed. Of these methods, those which depend on the oxidation of metallic phosphorus for the removal of the oxygen seemed to me to offer the greatest possibilities in economy, safety, and ease of operation.

Sellards,[1] the first to use such a method, devised an apparatus whereby he secured anaerobiosis in both plates and tubes. His apparatus was cumbersome, however, and consumed too much time in operation. Bushnell[2] modified this method by using an aluminum pressure cooker for the anaerobic chamber, securing anaerobiosis by burning phosphorus within the tightly sealed chamber. While rapid and economical, this also requires a piece of apparatus which is found in but few laboratories; hence, it has a limited range of use.

In the method described, the apparatus has been so designed that it can be made from inexpensive material to be found in all laboratories, or that can be readily constructed.

The apparatus, as shown in Fig 1, consists of a standard museum jar (*H*), into which is fitted a heavy wire frame (*G*), to hold the Petri dishes or tubes.

*From the Department of Bacteriology and Public Health, Washington University School of Medicine.

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The museum jar may be of any desired size. For Petri dishes, the five by twelve inch size is very satisfactory, accommodating from 10 to 15 plates of 10 mm. depth. The jar must not leak. It may be supplied with a closely fitting rubber gasket, or the lid may be ground in place, thus doing away with the necessity for a gasket.

The frame (*G*) is constructed of heavy steel wire, which is preferably about one-eighth inch in diameter. It is made just large enough to fit in the museum jar without jamming. It must be of such a height that the top of the frame reaches no higher than the bottom of the heavy flange about the mouth of the jar. About one inch from the bottom of the frame is soldered a platform, made preferably of galvanized wire gauze. When placed within the frame, the Petri dishes (*E*) rest on this platform and are thus kept out of the water which is used in the jar.

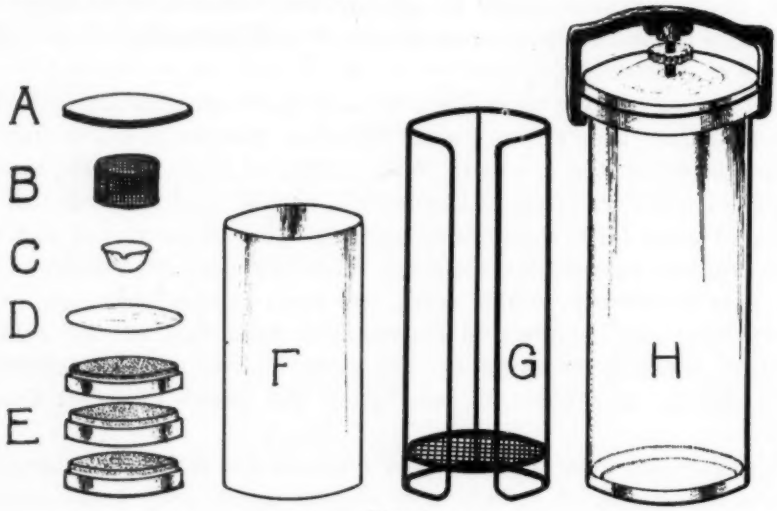


Fig. 1.

After the Petri dishes have been placed within the frame, a cylinder (*F*), made either of paper or tin, is placed over them. This is done to protect the sides of the Petri dishes or tubes from the phosphorus pentoxide vapors which form during the combustion of the phosphorus. The glassware is thus kept free from all deposit. A less satisfactory method of accomplishing the same result is to place a piece of filter paper (*D*) over each plate. If the cylinder is of tin, it should be closed at the top.

When the plates have been loaded into the frame, the tin cylinder is placed over them, and on top of this, resting on it, is placed a dish for holding the phosphorus. This may be of tin or porcelain. A small evaporating dish answers the purpose admirably, as does an empty ether can, cut in half. About this container is placed a screen (*B*), made of iron or nichrome wire gauze, which protects the sides of the museum jar from the burning phosphorus. A piece of asbestos board is cut to form a disc (*A*) of such size that it will fit on top of this gauze, and slip up and down freely within the frame. This permits of the use of a few or of many Petri dishes with the disc tightly in place over the gauze frame. In addition to the asbestos disc over the

screen, it is well to have another which rests on top of the frame, fitting snugly against the jar. This is an added factor of safety against the breakage of the jar or lid.

When the apparatus is assembled ready for use, about one-half inch of water is placed in the bottom of the jar. The water absorbs the phosphorus pentoxide vapor, formed during the combustion of the phosphorus. The Petri dishes or tubes are placed within the frame, and the tin or paper cylinder is placed over them. With the plates, evaporating dish, gauze, and asbestos disc in place, test the apparatus to see that the lid will fit tightly. Now thoroughly lubricate the mouth of the jar and the apposing surface of the lid with stopcock lubricant or a stiff mixture of vaseline and paraffin. It is also well to lubricate the rubber gasket. The latter should be put in place on the lid of the jar before loading the chamber with phosphorus.

To secure anaerobic conditions, place within the evaporating dish a piece of yellow stick phosphorus about one-eighth to one-quarter of an inch long. This is a sufficient charge for the five by twelve inch jar. Quickly place over the dish the asbestos disc (*A*), and see that it fits down snugly against the gauze surrounding the dish. Now clamp the lid into place. Within a few seconds the phosphorus takes fire and burns violently, establishing anaerobic conditions in the jar within a very few seconds. The heat given off by the oxidation of the phosphorus raises the temperature of the jar to approximately 40° C., so that an incubating temperature suitable for most anaerobes is quickly reached. This temperature will be maintained, with but a slight drop, if the apparatus is now put in the incubator. Left at room temperature, however, the heat is quickly dissipated, and the temperature drops to that of the room. When gelatin plates are to be used in the apparatus, if one first thoroughly chills the jar before adding the phosphorus, the final temperature within the chamber will not rise to a point sufficient to melt the gelatin.

This method has been used successfully in the cultivation of numerous mouth anaerobes for over a year, and no trouble has been experienced. It has been used successfully in the cultivation of many obligate anaerobes, very good results being secured in the case of the intestinal organisms. Surface colonies grow very well, and these may be examined under the microscope for colony form, thus facilitating diagnosis. Gelatin dilution plates are readily incubated in this apparatus, any evidence of liquefaction being observed in the original plate.

Certain precautions must be observed in the handling of the phosphorus to avoid premature combustion and danger of burns. Keep it in cool water at all times, being especially careful of this detail in summer. Unless this precaution is taken, burns or jar breakage may result. It is well to keep the phosphorus in the ice chest, wherever possible. Handle the material with forceps only, first making sure that they are cool. When opening the anaerobic chamber after incubation, immediately flood the unburned phosphorus with water, or remove the combustion dish to the hood with forceps. Any excess phosphorus may be recovered by melting under warm water, then

cooling. If the above precautions are taken, there will be no danger from burns or from phosphorus fumes.

Danger of breakage to the jar is obviated by the construction of the protecting screen and asbestos discs. Although the apparatus has been used more than a year by several bacteriologists, no breakage has occurred.

The moisture content within the jar is very high during incubation, thus aiding the growth of bacteria. Because of this, however, certain precautions must be taken to prevent the dropping off of the labels. These may be painted over with melted paraffin, or the articles may be marked with a blue wax pencil. India ink may also be used to mark the plates, but this is not as satisfactory as the wax pencil.

No trouble has been experienced with the phosphorus fumes injuring the growth. The most delicate anaerobes grow readily. A slight amount of elemental phosphorus is liberated, but if the plates are covered as described, this does not reach them. It has, however, no injurious effect on bacterial growth, as has been demonstrated not only from our own experience but also from that of Sellards and Bushnell as well.

This method possesses certain advantages:

1. All obligate anaerobes, even the most delicate, grow readily.
2. It is economical; the average cost of the phosphorus used for a dozen plates is from one-half to one cent.
3. It is rapid and certain. Anaerobic conditions are very quickly established, and if a slight leak occurs, the excess phosphorus will oxidize and completely remove all oxygen.
4. Both liquid and solid media cultures may be incubated in the apparatus. Loop dilution plates may be readily prepared.
5. There is no danger of contamination of the cultures at any stage of the process since the plate or tube remains unopened until ready for examination.
6. It retains its anaerobic conditions indefinitely and may be used for the storing of surface cultures.
7. By decreasing the amount of phosphorus used it may be used for the cultivation of organisms requiring a reduced oxygen tension.
8. A series of plates or tubes may be incubated under identical physical conditions, giving a good basis for comparison of cultures.

## SUMMARY

A new anaerobic method, depending on the oxidation of yellow phosphorus, has been described. In relation to its safety, ease of operation, certainty and cleanliness, it is superior to other methods. All anaerobes may be successfully grown, either in liquid or solid cultures, without danger of contamination. The average cost of incubation is less than one-twentieth of a cent per plate.

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# THE BOLTZ TEST: A TEST OF PRACTICAL VALUE IN THE DIAGNOSIS OF GENERAL PARALYSIS*

By Oswald H. Boltz, M.D., New York

IN 1923, I first described the above test under the title: "Studies on the Cerebrospinal Fluid with an Acetic Anhydride-Sulphuric Acid Test,"[1] and called attention to the frequency of a positive reaction with it upon the spinal fluids of certain cases of neurosyphilis, especially in general paralysis. Since then, however, two English observers have worked with the same test and have not only confirmed my investigations, but have also stressed the value of the test as a diagnostic aid in general paralysis. Both drew attention to the ease and rapidity with which our test could be performed, and, as a result of their findings, attested to its reliability in the diagnosis of general paralysis.

## THE METHOD OF PERFORMING THE TEST

Place 1 c.c. of fresh cerebrospinal fluid in a small glass test tube and to it add 0.3 c.c. of acetic anhydride. Shake the mixture well and then add, drop by drop, 0.8 c.c. of concentrated sulphuric acid. Shake the mixture gently once more. Then hold the test tube against a white background. The presence of a lilac tint indicates a positive reaction; a brown yellow, red yellow, or clear fluid is noted if the reaction is a negative one. The lilac color, characteristic of the positive reaction, may appear immediately after the addition of the sulphuric acid and, in slightly positive cases, may disappear in a minute or two; therefore one should watch the cerebrospinal fluid closely as soon as the sulphuric acid has been added, or a slightly positive case may be missed. In the majority of cases the lilac tint will remain for several minutes, occasionally for several hours.

The chemistry involved in the test, so far as I know, is not understood and awaits the research of a physiologic chemist.

In my original paper upon the subject, I concluded that our test was positive in all cases of general paralysis; that among psychiatric cases, it was predominantly positive in cases of neurosyphilis; that in psychoses not due to or complicated with syphilis, and of a functional nature, the test was negative; and that it differentiated two groups of tabetics: one giving a negative reaction, the other showing only a slightly positive one. In four cases of neurosyphilis with a negative spinal fluid Wassermann reaction, our test was positive.

In 1925, Grossman,[2] comparing the diagnostic merits of the Wassermann reaction with other simple tests used in the diagnosis of neurosyphilis, examined 60 cases which included 28 cases of general paralysis and one case of

*Read before the West Side Clinical Society of New York, April 8, 1926.
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locomotor ataxia. Using our test, he found that it was positive in all of his group of general paralytics, and negative in the one case of locomotor ataxia, as well as in the remainder of cases consisting of epileptics, psychoses due to arteriosclerosis, senile dementia, dementia precox, cases of melancholia, mania, and psychoses due to alcoholism. Grossman also found that the more active and advanced the syphilitic condition of the nervous system, the more positive was our test.

In 1926, Harris,[3] of the West Park Mental Hospital in Epsom, England, published an article in which he advocated the use of our test as a rapid, simple, and reliable test for confirming a clinical diagnosis of general paralysis. He called attention to the fact that the diagnosis of this disorder depends upon the recognition of certain mental symptoms, the eliciting of a number of physical signs, and the finding of certain definite changes in the cerebrospinal fluid. The serologic changes in the spinal fluid usually looked for to confirm a clinical diagnosis of general paralysis are: (*1*) a positive Wassermann reaction; (*2*) a paretic type of Lange colloidal gold curve (5555443211); (*3*) the presence of an increased amount of globulin; (*4*) a pleocytosis. Harris quite justly points out that, while a cell count and a globulin test on the spinal fluid can be performed with ease and rapidity and are of greatest value to the clinician who is often called upon to make a quick diagnosis, they alone do not furnish conclusive proof of the presence of general paralysis; that delay occurs until a laboratory report on the Wassermann reaction and the Lange colloidal gold curve are at hand. And furthermore, he believes that the discovery of a test which could be easily and quickly performed and which would be as reliable as the Wassermann reaction would be of considerable importance to the clinician, whether he were a consulting physician or a general practitioner. Using our test and comparing it with other serologic methods, Harris tabulates the results of his investigation in 180 cases as follows:

| | | |
|---|---|---|
| Of 92 cases of general paralysis the cerebrospinal fluid showed: | | |
| Wassermann reaction positive | 92 = | 100 per cent |
| Boltz test positive | 89 = | 97 per cent |
| Lange curve of paretic type | 84 = | 91 per cent |
| Increased amount of globulin | 90 = | 98 per cent |
| Increased number of cells | 91 = | 99 per cent |
| Of 5 cases of neurosyphilis (not general paralysis) the cerebrospinal fluid showed: | | |
| Wassermann reaction positive | 5 = | 100 per cent |
| Luetic Lange curve | 4 = | 80 per cent |
| Boltz test positive | 2 = | 40 per cent |
| Increased amount of globulin | 4 = | 80 per cent |
| Increased number of cells | 5 = | 100 per cent |
| Of 83 cases of other types of mental disorder the cerebrospinal fluid showed: | | |
| Wassermann reaction positive | 0 = | 0 per cent |
| Boltz test positive | 1 = | 1 per cent |
| Lange curve of positive type | 1 = | 1 per cent |
| Increased amount of globulin | 5 = | 6 per cent |
| Increased number of cells | 1 = | 1 per cent |

The above table shows that of 92 cases of general paralysis, a positive reaction with our test was obtained in 97 per cent. Harris states: "Of the three exceptions one was an undoubted case of general paresis with the typical mental symptoms, well marked physical signs, and positive cerebro-

spinal fluid findings. The other two cases, however, were by no means typical, and were probably very early cases in which there were few mental changes, some physical signs, and a slightly positive Wassermann reaction in the cerebrospinal fluid. In another case, a well-marked general paretic, the Wassermann reaction of the cerebrospinal fluid and the Lange curve were found to be negative at the first examination, but the acetic anhydride-sulphuric acid test (Boltz Test) was strongly positive, and the number of cells and the amount of globulin were slightly increased. A second specimen of fluid was examined a fortnight later, and this time a very faintly positive Wassermann reaction (+3) was obtained, the cells and globulin were slightly increased, the Lange test was negative, and the acetic anhydride-sulphuric acid test was again strongly positive. In this case, therefore, the acetic anhydride-sulphuric acid test (Boltz Test) was more sensitive than the Wassermann reaction." The same author found that the degree of positivity of our test in cases of general paralysis does not vary directly with that of the Wassermann reaction; that in a number of paretics who had undergone a course of malarial treatment our test showed no change, whereas the Wassermann reaction had in most cases become less strongly positive; that it appears to be more sensitive and reliable in general paralysis than the Lange colloidal gold test, since a positive result with it was obtained in 97 per cent of these cases, as against 90 per cent in the case of the Lange test. Of the 83 cases of other types of mental disorder not attributed to syphilis, only one case, a patient suffering from senile dementia, gave a faintly positive reaction with our test.

In a summary of the results of his investigations with the Boltz Test, Harris feels that it would seem justifiable to draw the following conclusions:

*1.* The Boltz Test upon the cerebrospinal fluid of cases of general paralysis is positive in almost every case (97 per cent), and hence is almost as reliable as the Wassermann reaction in this condition. *2.* It is negative in almost every other type of mental disorder except certain cases of neurosyphilis (other than general paralysis of the insane). *3.* It is a test which can be performed with great ease and rapidity and hence is of definite value to the clinician.

Both Grossman and I feel that the more active and advanced the syphilitic condition of the nervous system, as is the case in general paralysis, the stronger will be the reaction obtained with our test. In this organic disease, the nervous system is attacked from top to bottom by the Treponema pallidum and its destructive influence. It would seem, therefore, that the Boltz Test is an index of the destruction effected by the Treponema pallidum upon the tissues of the central nervous system. One might even be justified in assuming, perhaps, that it is of prognostic value in cases of neurosyphilis other than general paralysis. Of 21 cases of neurosyphilis (not general paralysis) in my series, 6 cases gave a slightly positive, 2 cases a positive, and 1 case a strongly positive reaction with our test, i.e., the test was positive in 42 per cent of neurosyphilitics who were not paralytics. In 5 cases of the same type studied by Harris, 2 cases (40 per cent) gave a positive reaction. To my mind it is quite possible that in those cases of neurosyphilis (not general paralysis) which gave a slightly or strongly positive Boltz Test, the

prognosis, from the standpoint of therapeutics, is not as good as in those who give a negative reaction. For the present, however, the prognostic value of the test still remains a problem to be checked up in clinics devoted to the treatment of neurosyphilis.

REFERENCES

[1]Boltz, O. H.: Am. Jour. Psychiat., July, 1923, iii, 111.
[2]Grossman: Jour. Ment. Sc., July, 1925, lxxi, 439.
[3]Harris: Brit. Med. Jour., January 23, 1926, No. 3395, p. 136.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**Lorber, J. A.: Simple Micro-Method for the Determination of Sugar in the Blood.** München. Med. Wchnschr., November, 1925, lxxii, 1921.

1. To 0.3 c.c. of serum or blood in a small centrifuge tube add 1.7 c.c. of distilled water and 0.5 c.c. of 10 per cent sodium tungstate.

2. Add 0.5 c.c. ⅔ N sulphuric acid.

3. Centrifuge and transfer 1.5 c.c. of the clear fluid to a graduated centrifuge tube.

4. Add 1 c.c. of Fehling's solution and "cook with steam" for 90 seconds.

5. Centrifuge, remove the supernatant fluid and to the residue of red cuprous oxide add 0.3 c.c. of nitric acid, 1 or 2 drops of hydrogen peroxide, and 0.2 to 0.3 c.c. of ammonium hydroxide.

6. Dilute to 1 or 1.5 c.c.

7. The copper-ammonium solution thus formed is compared with a similar standard solution of known concentration.

**Pinner, M.: The Daranyi Flocculation Reaction in Pulmonary Tuberculosis.** Am. Rev. Tuberc., December, 1924, x, No. 4, p. 441.

To 0.2 c.c. of serum add 1.1 c.c. of diluted alcohol (1 c.c. of 96 per cent alcohol to which has been added 4 c.c. of 2 per cent NaCl solution).

Mix and place in a water-bath at 60° C. for 20 minutes. Readings of the degree of flocculation are made at ½, 1, 2, 3 and 24 hours.

Flocculation in one-half to 1 hour is termed plus-four; after 2 hours, plus-three; after 3 hours, plus-two; and after 24 hours, plus-one.

A study of the reaction in 534 patients is reported, from which it is concluded that the reaction has little diagnostic value but appears to be of use in indicating the severity of the destructive process.

**Henley, R. R.: The Influence of Iron on the Growth of the Tubercle Bacillus upon Glycerinated Broth.** Am. Rev. Tuberc., November, 1925, xii, No. 3, p. 246.

During the course of experiments upon the possible inhibitory action of metals in metallic form upon the growth of B. tuberculosis, a striking difference was noted in the growth in media containing iron, the pellicle being heavier, more wrinkled, and more tenacious.

The medium used by the author as a routine for the culture of B. tuberculosis is a beef-infusion broth containing 1 per cent of peptone, 0.5 per cent of potassium biphosphate, and 7 per cent of glycerin by weight.

The reaction is adjusted with 2/N NaOH so that 100 c.c. of the medium requires 0.6 c.c. N/1 NaOH for neutralization with phenolphthalein. After titration and sterilization the broth has a PH of 7.1 to 7.3.

In contains naturally from 0.2 to 0.10 mg. of iron per 100 c.c.

Experiments were conducted to determine if the addition of iron salts exerted a favorable influence upon the growth.

Conclusions:

1. Glycerin-broth to which 20 mg. per 100 c.c. of ferric sulphate were added averaged 41 per cent heavier pellicle than broth without iron. Growth, however, is slower upon the iron broth during the first few weeks.

2. Growth is favorably influenced by ferric sulphate in media ranging in PH from 5.9 to 8-plus.

3. The favorable influence is due to the Fe ion and is not dependent upon the kind of iron salt added, ferric citrate, ferric sulphate, and ferric glycerophosphate all producing comparable effects when used in the proportion indicated above.

4. The most favorable concentration of iron appeared to be from 0.01 to 0.3 gm. of ferric sulphate per 100 c.c.

The rate of growth upon iron broth is at first lessened but in the later weeks the growth is more rapid and heavier than upon the control media.

The effect of increasing acidity and consequent change in the PH of the medium is less marked in broth containing iron, and the addition of iron in small amounts to glycerin-broth is therefore advantageous in the culture of B. tuberculosis.

**Malone, R. H.: A Method of Estimating the Degree of Solubility of Microorganisms in Bile and "Bile Salt" Solutions.** Indian Jour. Med. Res., April, 1923, x, 1144.

The method described by Malone for determining the degree of bile solubility of bacteria is based upon the principle that the opacity of a given bacterial suspension is directly proportional to the weight of the bacteria per unit volume of suspension.

The bacterial suspension is made from cultures grown on 5 per cent sheep blood agar slants for 20 to 24 hours at 37° C. The growth is suspended in normal saline, shaken thoroughly and filtered to remove clumps.

The removal of clumps may be neatly accomplished by twisting a small piece of sterile cotton around the end of a capillary pipette as in making a swab. This acts as an effective filter when the bacterial suspension is sucked into the pipette. The cotton should be removed before the suspension is blown out.

The clump-free suspension thus obtained is standardized against opacity tubes such as are used in the nephelometric standardization of vaccines. An acceptable concentration is approximately 2,800 million per centimeter of suspension (1.72 mg. of dry bacterial substance).

The solvent is prepared by dissolving one gram of sodium taurocholate in 1 c.c. of warm normal saline. One volume of this solution is added to nine volumes of bacterial suspension.

A set of standard opacity tubes, a capillary pipette of 1.5 to 2 mm. bore, internally lined and coated at the tip with paraffin, and small test tubes of the same bore as the opacity tubes (7 cm. long and about 7 mm. bore), are further required.

*Method.*—

1. Place one volume of sodium taurocholate solution in one of the small test tubes, using the capillary pipette.
2. Add nine volumes of bacterial suspension and mix.
3. Quickly determine the opacity by comparison with the standard tubes.
4. Incubate in a 37° C. water-bath for 30 minutes.
5. Remove from the bath, mix, and again read the opacity.

Tests should be made in duplicate and the readings, which should not vary more than one tube, are made to the nearest tube. Control tubes of the bacterial suspension are not required.

The test is thus recorded:

| | |
|---|---|
| Opacity of suspension | 10 |
| Opacity after addition of bile solution | 8 |
| Opacity after incubation | 2 |

The degree of solubility is, hence, recorded as 6.

**Capuani, G.: Alizarin Test in the Diagnosis of Tuberculosis.** Riforma med., April 13, 1925, xli, 345.

A known nontuberculous sputum is used as a control and diluted in a test tube in the proportion of one to five with distilled water.

A similar dilution of the specimen to be tested is made in another tube. To each

sample are added 20 drops of a 1 per cent alcoholic solution of alizarin. The tubes are stoppered and vigorously shaken and read after 20 minutes.

In tuberculous sputum a purplish precipitate occurs, the supernatant fluid being red.

In nontuberculous sputum, the entire tube is purplish red and becomes transparent within a few hours, and, if a precipitate forms, it is bluish and nubecular.

In Capuani's investigations the test proved neither specific nor conclusive, and he believes it to be of little value.

**Kessel, J. F., and Willner, O.: Some Clinical and Laboratory Aspects of Amebiasis with a Preliminary Report of Yatren Treatment.** China Med. Jour., May, 1925, xxxix, No. 5, p. 383.

The only certain method of laboratory diagnosis is by microscopic examination, and stools should be examined on six consecutive days before a negative report can be given.

A preliminary examination may be made by examining a smear in Donaldson's iodine-eosin stain: 5 per cent aqueous solution of potassium iodide saturated with iodine and mixed with an equal volume of saturated aqueous eosin.

The best method is to fix the smears in Schaudin's fluid for two minutes at 60° C. The formula for Schaudin's fluid is:

| | |
|---|---|
| Sat. aqueous solution of mercuric chloride | 200 c.c. |
| Alcohol, 95 per cent | 100 c.c. |
| Glacial acetic acid | 15 c.c. |

For the best results a special laboratory department should be created where the number of such specimens is large.

Very favorable results were secured with yatren treatment, which the authors believe will be a satisfactory therapeutic agent in both the acute and chronic forms of amebiasis.

**Jennings, J. E.: The Gram-Positive Anaerobes in Appendicitis and Its Complications.** New York State Jour. Med., January 16, 1925, xxv, No. 1, p. 1.

Jennings believes that the greater number of patients dying of peritonitis secondary to appendicitis die of an intoxication, and that the clinical manifestation of this intoxication is not unlike the classical descriptions of gas gangrene.

He reviews the literature relative to the presence of the gas bacillus in appendicitis, especially when associated with gangrene and reports his own studies in which, in gangrenous appendicitis, the Welch bacillus was frequently found both in the appendix and the peritoneal fluid.

Suspicious material is emulsified in sterile broth. A portion is inoculated into anaerobic milk tubes and another portion is injected into both lobes of the liver of a guinea pig, a slight laceration being produced by twisting the needle at the time.

The animal is then killed, placed in the incubator for two hours, and then autopsied.

The organisms may be demonstrated in smears and cultures from the liver, heart blood, and peritoneal exudate.

**Saxl, P., and Kelen, A.: The Trypsin-Flocculation Reaction in the Serum and Its Biological Significance.** Wien. Arch. f. inn. Med., November, 1925, No. 11, p. 549.

One gram of trypsin (Merck) is dissolved in 100 c.c. of normal saline containing 0.5 per cent tricresol; is allowed to stand one or two hours at room temperature, and is finally cleared by filtration. The tryptic activity, as tested by the digestion of casein, remains stable for two weeks if the solution is kept in the refrigerator.

Blood is collected before breakfast, and the serum is allowed to stand in the refrigerator for 24 hours.

A series of serum dilutions from 1:25 to 1:52,000 is set up in small (Wassermann) tubes. The last tube of the series is the trypsin control and contains only saline. To the first serum tube 1 c.c. of the 0.5 per cent tricresol-saline is added; to all the others 1 c.c. of trypsin-saline-tricresol solution.

All tubes are then incubated 24 hours at 37° C.

The serum control (Tube 1) is clear or slightly turbid; the trypsin control is clear, and the other tubes are either flocculated or distinctly turbid.

Normal serum always flocculates the trypsin solution. The reaction seems to be related to the trypsin content of the intestinal canal, decreasing and increasing with it.

It is suggested that the reaction may be of value in pancreatic conditions.

**Montenegro, J.: Cutaneous Reaction in Leishmaniosis.** Arch. Dermat. and Syph., February, 1926, xiii, No. 2, p. 187.

The growth of leishmania after 15 to 20 days on Nicolle's medium is washed off with normal saline. The suspension is twice washed with normal saline and recovered by centrifuging. Coca's fluid is added to the sediment from the last washing in sufficient amount to make a turbid suspension.

The liquid is then covered with toluol and allowed to stand at room temperature for three days. The container should be shaken daily to facilitate maceration.

The suspension is then centrifuged and the clear liquid—which is the extract used in the test—is pipetted off.

Before using it is cultured for sterility in gelatine at 37° C. and on Nicolle's medium at room temperature.

A sufficient quantity is injected intradermally to produce a wheal 8 mm. in diameter. This is usually about 0.2 c.c.

In positive cases redness, edema, and papule formation occur in 24 hours.

The alkaline Coca's fluid is injected as a control.

In 37 cases positive reactions were obtained in 32. No reactions were seen in 33 controls. Reactions were not obtained in cured cases.

**Prince, L. H.: A Rapid Stain for Nerve Tissue.** Bull. XI, Internat. Assn. Med. Museums, May, 1925.

Stain:

| | |
|---|---|
| Fuchsin f. bac. Gruber, sat. aq. sol. | 3–5 gtt. |
| Erythrosin Gruber 1 per cent aq. sol. | 15 gtt. |
| Methyl orange, Campbell and Bell, sat. aq. sol. | 40 gtt. |
| Aniline blue porous, Campbell and Bell, sat. aq. sol. | 25 gtt. |

Mix in the order listed and let stand 24 hours with frequent agitation during this period. No precipitates are formed and the solution appears to be stable so that multiples of the unit may be prepared if desired.

Investigations are now in progress to perfect this combination with American dyes.

Tissues are fixed in formalin and cut in paraffin.

Sections are passed through xylol, 95 per cent alcohol and water to prepare for staining.

The excess of water is drained off and the slide covered with the stain for 10 to 30 seconds. It is then washed in running water, dehydrated in absolute alcohol (copper sulphate-dehydration is satisfactory), blotted carefully but quickly, cleared in xylol, and mounted in balsam.

Several sections should be stained simultaneously and removed at intervals of 5 to 60 seconds in order that the reactions of various elements to the stain may be noted.

Staining reactions:

Nerve cells in general: light to dark purple with deeply stained Nissl bodies.

Normal cells: nuclei blue: nucleolus orange-red.

Usually deeply stained in 20 seconds.

Myelin: pink to deep red, especially if this substance is granular. The axis cylinder is almost black, tending to orange as disintegration progresses.

Neurolemma: light or dark blue.

Neuroglia: blue.

Erythrocytes: orange to red orange.

Lymphocytes: pale blue.

Corpora amylacea: pale blue.

After the section has been taken from the first alcohol it may be treated for 10 to 20 seconds with 1 per cent glacial acetic acid in 90 per cent alcohol. The stain is then applied without previous washing of the section in water.

Marked intensity in staining of some of the elements is secured, especially in neuroglia and connective tissues and myeln. Nerve-cell detail, however, may be obscured if the staining is too long.

**Sheridan, W. F.: A Rapid Hematoxylin-Eosin Staining Method for Paraffin Sections.** Bull. XI, Internat. Assn. Med. Museums, May, 1925.

Harris' hematoxylin stain is used and staining may be completed in five minutes.

Sections should not be over 5 micra in thickness and should be mounted with albumin.

*Method.*—

1. Xylol 2 minutes.
2. Rinse with 95 per cent alcohol (from drop bottle).
3. Hematoxylin, Harris, 2 minutes.
4. Rinse with 95 per cent alcohol.
5. Acid alcohol (1 per cent HCl in 70 per cent alcohol), 15 or more seconds.
6. Rinse with 95 per cent alcohol.
7. Ammoniated alcohol until rose color is replaced by blue: about one minute (freshly prepared before using: stronger ammonia 4 drops, alcohol 95 per cent 50 c.c.).
8. Rinse with 95 per cent alcohol.
9. Eosin (0.25 per cent in alcohol 95 per cent) 30 seconds.
10. Rinse with acetone from drop bottle. Acetone should be water-free and should not give more than a faint turbidity with xylol.
11. Xylol—clearing is instantaneous.
12. Mount in balsam.

**Wearn, J. A., and Richards, A. N.: Quantitative Estimation of Minute Amounts of Urea.** Jour. Biol. Chem., November, 1925, lxvi, No. 1, p. 275.

The method described was devised for the estimation of the urea content of glomerular urine in frogs and suffices to measure, with a probable error of 10 per cent, as little as 0.0005 mg. of nitrogen in the form of ammonia or urea.

The method consists in hydrolysis of the urea by heating with acid at 150° C. simultaneous nesslerization of the unknown and a known ammonium sulphate solution, and quantitative color comparison.

All reagents, of course, must be ammonia-free.

The tubes for color comparison were 8 cm. in length with an inside diameter of 5 mm. and were calibrated at 1 c.c. Readings were made by artificial light diffused through "daylight" glass, the tubes being held in a rectangular rubber mat to exclude all light not passing through the tubes.

The method is described as tested on known solutions of urea and ammonium sulphate, the results obtained with glomerular urine being withheld until a sufficiently large series accumulates.

From twice recrystallized urea a solution containing 0.2 mg. N in 1 c.c. was prepared.

A small amount (1 to 3 mg.) of this solution was drawn up in a capillary tube and discharged into a weighed capillary tube.

After weighing the tube and its contents, the fluid is transferred to a clean, dry, hard glass tube (pyrex) 6 inches long, inside diameter 5 mm., closed at one end and graduated at 1 c.c.

Enough normal HCl is added to make the concentration approximately 0.1 N. The upper end of the tube is sealed in the flame and tube autoclaved one hour at 150° C.

The top of the tube is then broken off and the inner surface of the top is rinsed with a few drops of water into the main bulk of the fluid in the larger fraction of the tube.

A series of comparative tubes are then charged with varying amounts of standard ammonium sulphate solution (1 c.c. equals 0.002 mg. N) the quantities chosen (0.1 to 0.3 c.c.) being such as to include the assumed nitrogen content of the specimen to be examined.

Normal HCl is then added to make the acid content exactly equal to that of the urea solution.

Then 0.2 c.c. of Nessler's solution is added to each standard tube and to the hydrolyzed urea solution. The contents of each tube are diluted to 1 c.c. and comparison made in the comparator.

With the method described the error was less than 10 per cent.

**Maximon, A. A.: Role of the Nongranular Blood Leucocytes in the Formation of the Tubercle.** Jour. Infect. Dis., November, 1925, xxxvii, No. 5, p. 418.

Maximon reports his studies upon the histogenesis of tubercle through the observation of tissue cultures inoculated with tubercle bacilli.

Rabbit leucocytes were obtained by centrifugation in a paraffined centrifuge tube and were coagulated by the addition of a few drops of embryonic extract. This coagulum was washed in Ringer's solution, cut into small pieces with sterile precautions and planted in dilute rabbit blood plasma and embryonic extract from 13- to 16-day rabbit embryos. Immediately after clotting of the plasma drop containing the explanted white blood layer, the edge of the fragment was inoculated with a culture of human tubercle bacilli grown on glycerol agar. The cultures were observed for seven days, being transplanted into fresh medium every second day. Observations were made of the living culture and after Zenker-formal and celloidin preparation of serial sections.

It was shown that the epitheloid and giant cells of tuberculous lesions have the same origin as the polyblasts or mononuclear exudate cells in common or purulent inflammation.

They arise partly from local fixed elements—histocytes, and partly from nongranulated blood cells, lymphocytes and monocytes.

The origin of epitheloid cells varies in accordance with the circumstances of the case, that is, the type of cells available. A sharp discrimination between monocytes and lymphocytes in their rôle in the formation of tubercle cannot be made. The monocytes respond more promptly and sooner reach the fully developed epitheloid stage, but the lymphocytes follow the same path.

The paper is illustrated with a color plate.

**Pinner, M.: Complement-Fixation in Tuberculosis, III. Studies on the Nature of the Antigen.** Am. Rev. Tubere., October, 1925, xiii, No. 2, p. 142.

Pinner reports a study of complement-fixation in tuberculosis with more than 70 different antigen extracts prepared according to various methods which have been described.

Each antigen was tested, under identical conditions, against a mixture of 3 or 4 positive sera, and the antigenic quotient of each extract was determined by the method described by Kolmer.

He concludes that the main complement-fixing substances are closely associated with that fraction of the bacilli which is practically insoluble in ether, acetone and chloroform and soluble in alcohol. There is no satisfactory proof that the alcohol-soluble substances contain the only antigenic principle but no extract from which these substances have been removed furnishes a satisfactory antigen.

The degree of dispersion plays an important rôle in the antigenic value of lipoidal tuberculosis antigens.

The protein substances exert an inhibiting influence upon the lipoids in complement-fixation.

There is no evidence to indicate that certain tuberculous sera react only with protein and others only with lipoidal antigens.

**Pinner, M.: Complement-Fixation in Tuberculosis, III. Studies on the Nature of the Antibody.** Am. Rev. Tuberc., November, 1925, xii, No. 3, p. 233.

By some authors the complement-fixing bodies in tuberculosis are held to be associated with the globulin fraction, and by others, with the lipoidal fraction of the serum.

Pinner studies this question anew by digesting positive sera with trypsin and dialyzing them until chemically free from proteins and protein split products. Such preparations were found to contain complement-fixing bodies. They are not, therefore, globulins and must be either lipoids or proteins not broken up by trypsin. True liposes with a specific action on tubercle bacillus lipoids could not be found in sera containing complement-fixing bodies.

Complement-fixing bodies are readily destroyed by ether and any procedure including ether extraction is to be excluded from examinations for complement-fixing bodies in tuberculosis.

**Pinner, M.: Complement-Fixation in Tuberculosis with Wassermann's Antigen.** Am. Rev. Tuberc., April, 1925, xi, No. 2, p. 139.

In March, 1923, Wassermann introduced an antigen for tuberculosis complement-fixation tests, results of a trial of which are reported by Pinner.

Tubercle bacilli are extracted at incubator temperature with tetraline (a tetrahydrated naphthaline) in a shaking machine. After several weeks the bacterial residue is nonacid-fast. To this material lecithin is added. The preparation of the antigen is not further described, reference being made to Wassermann's original paper (Deutsch. med. Wchnschr., 1924, xlix, 303).

Using this antigen 646 patients were tested, 71.5 per cent of positive reactions being obtained.

A number of false positive reactions were obtained in nontuberculous patients and in pregnancy.

Pinner concludes that the test with Wassermann's antigen has limited diagnostic value and no prognostic significance.

**Friedenwald, J., and Morgan, Z.: Observations on the Rosenthal Phenoltetrachlorphthalein Test as a Means of Determining Liver Function.** Ann. Clin. Med., November, 1925, iv, No. 5, 415.

A report of results with this test in various conditions.

In catarrhal jaundice marked retention was noted, persisting for some time after disappearance of the icterus and thus suggesting a distinct disturbance of the liver cells.

In cirrhosis of the liver the findings were variable.

Definite retention was noted in all cases of hepatic cancer; in syphilis of the liver the degree of retention is related to the degree of involvement.

In affections of the gall bladder in which the liver is not involved, retention does not occur.

As an addendum, the authors report that, in their limited experience with it, the bromosulphthalein test is equally valuable and has many advantages.

**Greene, H. M.: Lumbar Puncture and the Prevention of Postpuncture Headache.** Jour. Am. Med. Assn., Feb. 6, 1926, lxxxvi, 391.

Greene is convinced that postpuncture headache is due to loss of fluid by leakage which eventually leaves the brain without a water cushion.

He uses, therefore, a needle of small caliber, the point of which is rounded, tapering, and sharp rather than blunt and cutting, thus avoiding any severance of the fibers of the spinal dural sac and reducing leakage to a minimum. These special needles were made by the Becton, Dickinson Co.

His further precautions are:

1. The patient is placed upon the table in a comfortable position and—this being an essential point—should remain motionless while the needle is in the dural sac.

2. A local anesthetic should be used in both the skin and deeper tissues.

3. A special needle as described, gauge 22, is used.

4. After fluid appears, the sample should be aspirated.

In 215 consecutive punctures so performed, headache occurred only in two, in many instances the puncture being done at noon and the patient returning to work without ill effect.

**Berman, L.: The Diagnostic Criteria of Chronic Parathyroid Insufficiency with Special Reference to the Phosphate Content of the Blood.** Am. Jour. Med. Sc., February, 1926, clxxi, No. 2, p. 245.

Berman thus tabulates the criteria of value in the diagnosis of this condition and as gauging the effect of treatment:

1. Dystrophies of the hair, nails, teeth and skin.

2. Mechanical hyperexcitability of the nerves: Trousseau and Chvostek phenomena.

3. Electrical hyperirritability of the peripheral nerves.

4. Diminished calcium content of the blood.

5. Decreased phosphate content of the urine and phosphate retention in the tissues with increased phosphate content of the blood.

In twenty cases the blood calcium was within the normal limit of 9-11 mg. per 100 c.c.

In all but one, however, there was a definite increase in the inorganic phosphate of the blood, one showing 6 mg., nine having over 5 mg., and the average amount being 4.89 mg. per 100 c.c. The blood calcium averaged 10.2 mg.

In 37 nonparathyroid controls the calcium averaged 10.9 mg. and the blood phosphates 3.96 mg. per 100 c.c.

**Vigneaud, V., and Karr, W. G.: Carbohydrate Utilization. I, Rate of Disappearance of d-Glucose from the Blood.** Jour. Biol. Chem., November, 1925, lxvi, No. 1, p. 281.

In view of the clinical significance attaching to the rate of disappearance of glucose from the blood by means of tolerance tests, the authors comment that but little is known of the mechanism involved and report studies upon various factors of possible influence.

Their studies were made upon rabbits.

Further evidence is offered that this mechanism is practically independent of the rate of absorption from the intestine.

Fasting invariably decreases the rate of disappearance of glucose following a hyperglycemia from ingestion of glucose.

After a period of fasting, specific foodstuffs vary the rate. Proteins 4 hours before or with the glucose test meal have little or no influence; 18 hours before, greatly decrease the rate. Fat given 18 hours before also decreases the rate of disappearance. Glucose given 18 hours before the glucose test meal slightly increases the rate.

Adrenalin 18 hours before greatly increases the rate of disappearance; morphine, although producing a hyperglycemia, produces no increase.

A glucose meal immediately after an insulin hypoglycemia has returned to normal shows a decreased rate of disappearance.

Sodium bicarbonate during a fast prevents the decrease but blood PH and $CO_2$ studies show that acidosis is not a predominant factor in causing this change during a fast.

These studies indicate the need of controlling the diet, period of fast, and medication before performing tolerance tests.

**Forster, E.: The Spirochetes in Malaria-Treated Paralytics.** München. med. Wchnschr., Dec. 18, 1925, li, 2197.

Three patients were studied. Material withdrawn by brain puncture was examined by dark-field illumination. Spirochetes were found in two of the cases. In one they were very numerous and actively motile; in the second case only two spirochetes were found after prolonged search.

The material was then embedded and sectioned.

All three cases showed definite histologic changes.

In the patient in whom numerous organisms were found they were seen in the capillaries. The ganglia cells were markedly altered.

The report, though covering only three cases, is made because of previous reports that in malaria-treated cases spirochetes could not be demonstrated. It is suggested that this may be due to the uncertainty of staining methods for spirochetes.

**Pucher, G. W., and Burd, L. A.: Note on the Stability of Dakin's Solution.** Buffalo Gen. Hosp. Bull., April, 1925, iii, No. 1, p. 20.

The bleaching powder used in the preparation of Dakin's solution must contain at least 25 per cent available chlorine. The boric acid and sodium carbonate employed for PH adjustment must be free from organic impurities. For storage, dark, amber colored bottles must be used. These are washed clean from organic matter and are filled with Dakin's solution. After standing for 24 hours this solution is discarded and the bottles are filled with fresh diluted solution for use. If stored at 10 to 25° C. such solutions are stable for at least six weeks.

**Knowles, F. C., and Decker, H. B.: Gastric Acidity in Acne Vulgaris.** Arch. Dermat. and Syph., February, 1926, xiii, No. 2, p. 215.

There is a consensus of opinion that acne vulgaris is frequently accompanied by gastrointestinal disturbances; a study of the gastric acidity was made in 25 cases.

Ten cases showed a total acidity below 50, seven between 50 and 60, four between 60 and 70, and four over 70.

There was no relation between the acid values and the duration, type, or severity of the condition. When the acidity was low, improvement occurred under the administration of dilute HCl.

**Cumming, W. M.: The Serology of the Bovine Type of the Tubercle Bacillus.** Tubercle, London, December, 1925, vii, No. 105, p. 8.

The investigation herewith reported was undertaken to verify the contention that human and bovine tubercle bacilli are serologically identical.

Antihuman tubercle serum was obtained by the immunization of a rabbit with an emulsion of ether-acetone extracted human tubercle bacilli from a broth culture. The titer was 1:1600.

More difficulty was encountered in preparing an antiserum with a similar preparation of bovine bacilli, but one was finally secured with a titer of 1:800.

Emulsions were prepared from cultures on Dorset's or Lubenau's egg medium, extracted with acetone and ether, dried, ground to a paste with 1:4 sodium bicarbonate, and suspended in normal saline with 0.5 per cent phenol, the suspensions being standardized to contain 32,000 million organisms per centimeter. One c.c. of this suspension and 1 c.c. of antiserum in a strength equal to 16 times the titer were mixed and allowed to stand overnight at room temperature.

After centrifugation, the supernatant fluid containing the antiserum in eight times the titer, was distributed into three sets of four tubes: into the first set was distributed 0.25 c.c. of unabsorbed serum so diluted that strengths equal to 8, 4, 3, and 2 times the titer were obtained, and 0.25 c.c. of a 2000 million per c.c. bacillary suspension. The final serum concentrations were thus 4, 2, 1.5 and 1 times the titer, acting upon 500 million organisms.

Similar volumes and dilutions of absorbed serum and 0.25 c.c. of a 2000 million per c.c. suspension of homologous organisms were added to the second set, and 0.25 c.c. of a 2000 million per c.c. suspension of bacilli to be tested was added to the third set.

In addition, 0.25 c.c. of the suspension of test organisms was set up against 0.25 c.c. of 1:12.5 dilution of normal rabbit serum.

The tubes were then incubated 24 hours at 56° C. in an oven or water-bath.

The experiments showed that tubercle bacilli of human and bovine types are not distinguishable by agglutination or absorption of agglutinins.

# *BOOK REVIEWS*

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building. Richmond, Va.)

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## *Organic Derivatives of Antimony**

THIS volume is one of the American Chemical Society's Series Service of Scientific and Technologic Monographs. Some twenty-five or more of these have already been published. It is hoped that "two rather distinct purposes may be served by these monographs. The first purpose, whose fulfillment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended."

The preparation of the present monograph was undertaken in connection with a study of antimonials which is being made in the Departments of Pharmacology and of Tropical Medicine of the Harvard Medical School.

The chemistry of organometallic compounds has been expanding very rapidly during the last few decades, owing largely to the marked physiologic activity of these substances. In this great class of compounds there are, on the one hand, some of the most powerful of the war gases and, on the other hand, some drugs which are indispensable in modern therapeutics. Although the organic arsenicals have been investigated much more thoroughly than the antimonials, studies in the latter field are being carried on quite actively in connection with researches on protozoal diseases because it appears that in certain of these diseases the antimonials are much more efficacious than the arsenicals.

The author has attempted to collect all the available literature on the subject and to present it in such a way that it will be useful both for those desiring a general knowledge of the organic derivatives of antimony and for those embarking on researches in this field. To this end the material has been so arranged that the first chapters are devoted to general discussions without the introduction of detailed directions for the production of the various compounds. The latter type of detailed information for the laboratory production of the various compounds is presented in the last chapters.

*Organic Derivatives of Antimony. By Walter G. Christiansen, Instructor in the Harvard Medical School. Cloth. Pp. 230. The Chemical Catalogue Company, Inc., New York.

The book contains ten chapters of which the sixth, written by Dr. George C. Shattuck, deals rather briefly, but in an interesting manner, with the use of organic antimonials in trypanosomiases, the leishmanioses, the schistosomiases, granuloma inguinale and leprosy. It also takes up the action of antimonials in the body and the methods of treatment with antimonials.

The book is a valuable contribution and will be of much use to those carrying on special investigations in chemotherapeutics or in the field of organometallic compounds.

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## EDITORIALS

### *The Measurement of the Efficiency of the Circulation*

A SATISFACTORY and practical laboratory method for the estimation of the efficiency of the heart in carrying on the circulation has not as yet been perfected. There is great need for such a procedure. The method of Henderson and Haggard,[1] using ethyl iodide, gives promise of being satisfactory. The principle is that the rate of absorption of a relatively slightly soluble gas, such as ethyl-iodide vapor, from the alveolar air into the blood is nearly proportional to the circulation through the lungs. The technic of the procedure is claimed to be simple, accurate, and reliable. The patient is made to inspire dilute ethyl-iodide vapor for ten minutes through a specially devised apparatus. Air samples are taken to determine: (*1*) the volume of air breathed per minute; (*2*) the concentration of ethyl iodide in the inspired air; (*3*) the concentration in the expired air; (*4*) the concentration in the alveolar air.

(*A*) The amount of ethyl iodide absorbed per minute is determined by multiplying factor *1* by the difference between *2* and *3*. (*B*) The amount of

ethyl iodide in the arterial blood is equal to the factor *4* multiplied by the coefficient of solubility of the gas (approximately 2.0). Then the volume of the blood flow through the lungs is determined by dividing *A* by *B*. The complete determination of the respiratory exchange, with oxygen consumption as a basis for indirect calorimetry, that is, the basal metabolism, is made simultaneously. One operator can carry out the procedure, including the determination of the minute amounts of ethyl-iodide vapor, by the accurate iodine-pentoxide method and also the analyses for oxygen and carbon dioxide in considerably less than an hour. The ethyl-iodide vapor has a conveniently low air blood distribution coefficient, approximately 2.0, at body temperature. The ethyl iodide is furthermore completely decomposed in the tissues so that the amount returning to the lungs in the venous blood is practically negligible. Further advantages claimed for the method are that it is applicable to practically all conditions and requires practically no cooperation upon the part of the patient other than to breathe as naturally as possible through a mouthpiece and valves for ten minutes.

J. W. Moore, using this method with a modification of apparatus so perfected that he could measure foot-pound work with the patient lying in bed and after exercise, had hoped to be able to determine even the extent of the lowering of the circulation efficiency in patients with heart disease and establish the limit of activity that a patient might safely engage in. He recently reported, however, only disappointment and apparent failure of the method at the American Medical Association meeting in Dallas. The method still deserves consideration and further attempts to apply it chemically.

Burwell and Robinson[2] have devised a more elaborate method based upon the principle of Fick. Calculation of the cardiac output by Fick's principle requires an accurate estimation of: (*1*) the amount of oxygen absorbed by the body per minute, and (*2*) the amount of oxygen taken up by each unit of blood as it passes through the lungs. Division of the total oxygen per minute by the oxygen taken up by each unit of blood will express the number of units of blood passing through the lungs each minute. The determination of the oxygen taken up by each unit of blood requires accurate estimation of: (*1*) the oxygen content of the blood as it leaves the lungs (arterial blood), and (*2*) the oxygen content of the blood as it enters the lungs (mixed, venous blood). The corresponding figures for carbon dioxide would serve as well in the application of Fick's principle. The gas content of the mixed venous blood can be approached only indirectly in man.

By a respiratory method, properly diluting the oxygen of the lung air by inspiring a 0.7 to 2 per cent oxygen mixture before each rebreathing, the gas mixture ($O_2$ and $CO_2$) in the rebreathing bag may be promptly brought into equilibrium with that of the mixed venous blood entering the lungs. Thus the lungs are used as an aeronometer. A tonometer is filled directly with this rebreathed gas mixture and peripheral venous blood of the subject is equilibrated with it under standard conditions. The peripheral venous blood so treated is, as it were, "artificial," mixed, venous blood, and the analysis of this for $O_2$ and $CO_2$ by the method of Van Slyke and Stadie, sup-

plies the desired data. The measurement of the output of the heart from this data, obtained by the application of the method described based upon Fick's principle, is accomplished as follows: $M = \frac{O}{W} \times 100$. $M$ = minute output of the heart in cubic centimeters. $O$ = c.c. of oxygen absorbed per minute. $W$ = volumes per cent of oxygen utilized. As the gases of the blood are now commonly calculated as volumes of gas per 100 c.c. of blood, the formula can be conveniently employed.

The established normal for adults at complete rest varied from 3,500 c.c. per minute and 58 c.c. per beat to 6,800 c.c. per minute and 103 c.c. per beat. These figures lend support to the view that the output of the heart per beat is not fixed but variable. Further observations bearing upon this are anxiously awaited. The method, though complicated, is the most promising that has thus far been put forth. It may lead to a simpler clinical laboratory method of this most important quantitative function of the body, the efficiency of the cardiorespiratory system.

The clinical methods of estimating cardiac function are still quite crude, but standardized by some such exact method and elaborated by electrocardiographic and roentgenographic studies, the ordinary exercise tolerance tests may become increasingly valuable to the clinician as a means of establishing rational prognosis.

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—*G. R. H.*

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**References. (Read these.)**

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